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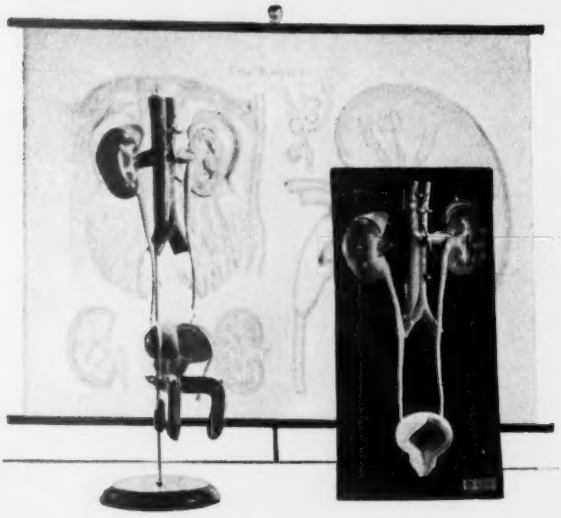

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THE CASE FOR LIVE POLIOVIRUS VACCINATION**

Large scale programs for the control of poliomyelitis through the use of active immunization can be said to have started, both in the United States and elsewhere, in 1955. This step marked the end of a 40-year period in which experimental efforts to vaccinate against poliomyelitis had been repeatedly tried. As early as 1910, Dr. Simon Flexner, the Director of the Rockefeller Institute, and at that time the leading United States authority on the virus etiology of poliomyelitis, is quoted in a newspaper as having referred to Landsteiner and Popper's recent (1908) discovery of the virus of poliomyelitis[13] in the following terms: "Now that the virus of poliomyelitis has been discovered, the development of a vaccine to prevent the disease should only be a matter of months." There is no proof that Dr. Flexner ever made such a statement, but his contemporary colleagues do recall his eager hopes and it is unlikely that he (or they) suspected in 1910 that it would be a matter of some 540 months before such a prediction was to be realized in any true sense of the word, and, indeed, the search for an ideal vaccine may not yet be finished.

The many interim, albeit sporadic, attempts to develop anti-poliomyelitis vaccines are fairly well known and started with experimental work in monkeys in which both killed and live virus vaccines were tried. The first of the trials in man were those with ricineolated and formalinized vaccines. They were not particularly successful. It was not until a few years after the composition of the poliovirus family, consisting as it does of three different serotypes, became known[18] that Dr. Salk, working under the auspices of the National Foundation for Infantile Paralysis, began his work on a formalinized (killed) poliovirus vaccine. By 1954 the vaccine was ready for a large-scale field trial known today as the Francis field trial.[4] From that date forward, the control of poliomyelitis became a reality.

Since 1955, therefore, the record has been impressive, and the prevalence of paralytic poliomyelitis has been appreciably reduced within areas where

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the Salk-type vaccine was adequately used.[33] * It is the only vaccine at present approved as a method for the prevention of paralytic poliomyelitis in the United States. Its potency has been improved recently, and the gains which have been made in reducing the incidence of paralytic poliomyelitis are almost certain to continue. On these bases the Salk-type vaccine deserves vigorous support, and campaigns to extend its use are distinctly in order. Nevertheless, as with most such prophylactic and therapeutic measures, techniques are liable to change. Thus there are questions not only with regard to the ways of giving the Salk-type vaccine but also as to whether it will be eventually replaced by a live poliovirus vaccine, or conceivably whether there will be some sort of combination of the two.[34] The ultimate aim is, and has been, of course, to reduce paralytic poliomyelitis to the lowest possible minimum.

The case for the *live* poliovirus vaccine rests in part upon the idea that an actual induced infection will produce a more solid type of immunity than will the parenteral injection of a killed viral antigen. This suggests that the Salk-type vaccine is not quite adequate and so one should first look at the record of the past four years in which the Salk-type vaccine has been used in the United States. In some areas in 1959 the efficiency of the killed vaccine in protecting against paralytic poliomyelitis has been rated as high as 90 per cent. And yet troublesome epidemics of poliomyelitis occurred in certain U. S. cities in 1958 and 1959, the latter having been a relatively bad year with more than 5,000 paralytic cases as of December 1959. Before criticizing the potency of the killed vaccine, it should be pointed out that the major reason why paralytic poliomyelitis has not been more markedly reduced, has been the failure, during the past four years, of many to take advantage of the vaccine for themselves or for their children. The vaccine itself cannot be blamed for this. In many U. S. cities not more than half the population under 40 years of age has been vaccinated and in the serious U. S. epidemics of 1958 and 1959 most of those who contracted paralytic poliomyelitis either had not been vaccinated at all or had been inadequately vaccinated. For example, in the Detroit epidemic of 1958, only 12 per cent of the paralytic cases occurred in persons who had received three doses of vaccine, whereas 73 per cent of such

* Current techniques for the administration of this vaccine call for its intramuscular inoculation in three divided doses of 1 ml. each, the second being given one month to six weeks after the first, and the third not earlier than seven months after the second. To increase the efficiency of the vaccine, the Surgeon General of the U.S. Public Health Service recommended in June 1959 that a fourth dose might be given one year after the third. There has even been some discussion as to the possibility of recommending a subsequent *annual* dose to children, but no definitive opinion has been reached on this point which is for the future to decide.

cases occurred in persons who had received no vaccine.[19] In particular, it has been among infants and young children below the age of six years and young adults between 18 and 40 where the vaccination program has lagged. To meet these deficiencies in the United States much attention is being brought to bear on this problem by municipal, state, and national health services.

In reviewing indications for the use of the Salk-type vaccine one should consider the way that it is supposed to work, namely, that by inoculating killed poliovirus into the body a certain degree of immunity is created which protects the person against a subsequent *severe* infection with live, virulent poliovirus. One should recall in this connection that ordinarily almost 99 per cent of natural infections with live virulent poliovirus are so mild as to escape notice. This is the way (i.e., from repeated mild, inapparent infections) that most people before the days of vaccination gained their immunity. In some persons, however, the infection with poliovirus is severe enough to cause paralysis and this is the risk that unvaccinated people take. Thus the Salk-type vaccine does not prevent *mild* infections with polioviruses. Its effect is to keep the immunity of the person who has been vaccinated at such a level that when he does become infected, his "case" will be mild.

Live poliovirus vaccines. Of growing interest in the world-wide campaign to control paralytic poliomyelitis has been the live attenuated poliovirus vaccine.[10] The history of live poliovirus vaccines goes back almost 40 years, as far as experimental work in monkeys is concerned. In man it goes back almost a decade. In this search Dr. Hilary Koprowski, at that time an associate of the Lederle Laboratories of Pearl River, N. Y., was a pioneer.[11] His early observations paved the way for much future work by himself and others.[51] Since 1952, a number of candidate strains representing the three serotypes of polioviruses have been proposed and several have been tried out on a large scale. The major investigators and proponents in these developments besides Dr. Koprowski have been Dr. Albert B. Sabin of the Children's Hospital Research Foundation,[17] University of Cincinnati Medical School, and Dr. Herald Cox of the Lederle Laboratories.* Perhaps one of the reasons why the idea of a live poliovirus vaccine found favor with certain immunologists has been the satisfactory experience with live virus vaccines which has been encountered in the field of veterinary medicine. It is also an acknowledged principle that, by and large, a more solid type of immunity can result from an actual

* See reference 10, and many papers by H. R. Cox, B. J. Cabasso, S. S. Markham, Roca-Garci, and others.

infection than from the injection of a killed substance into the body. Obviously, this last statement needs qualification, because one could continue injecting killed substances into a given person, month after month or year after year, thereby producing a very solid kind of immunity, but such a procedure would not be very practical. The problem then in the program for the control of paralytic poliomyelitis is to see, first, whether the domesticated (so-called attenuated) polioviruses now being proposed to be used as vaccines by Dr. Koprowski, Dr. Sabin, and Dr. Cox are safe; and, secondly, whether they will confer a solid kind of immunity on the person thus vaccinated—at least as solid if not more so than that produced by the killed poliovirus vaccine.

The principle of this type of vaccination is obviously similar to that of vaccination against smallpox, namely, by inducing an actual though harmless infection with a live, a virulent virus, related to that which causes the serious disease, one also induces immunity to the serious disease. As the natural portal of entry of poliovirus is through the alimentary tract, the live poliovirus vaccines are administered by mouth instead of by injection. Thus they might serve as agents which should be more easily administered. They will probably be less expensive. However, whether or not they are practical and more efficient remains to be seen.

There is evidence that the kind of immunity induced by the killed Salk-type vaccine may differ from that of the live poliovirus vaccines. With the former it is assumed that adequate neutralizing antibody levels are a measure of the immunity produced and that they reflect something that limits the spread of poliovirus within the body, thereby preventing significant invasion of the central nervous system. There is less evidence, however, that such circulating antibodies are a measure of resistance against subsequent implantation of the virus or penetration of the virus through the superficial or deeper layer of the integument of the alimentary tract. Thus the presence of artificially induced antibodies of moderate levels in itself does not prevent subsequent alimentary infection. With the live poliovirus vaccines, antibodies are also produced which circulate in the blood stream, but evidence suggests that in addition there is produced some kind of tissue resistance, or locally concentrated humoral resistance, to which the term "local resistance" might be applied until a better name is suggested. In any event, a person vaccinated (i.e., actually infected) with live attenuated poliovirus is more apt to have developed a state of resistance for the prevention of a reinfection with virulent polioviruses than is the person who has received killed virus. Re-infections nonetheless do occur.

So far the attenuated poliovirus vaccines have certain advantages, but there are complexities in their use still to be unravelled and they are still

to be regarded, as far as the U. S. Public Health Service is concerned, as being in an experimental stage. One feature is that the live virus vaccine does not always take and it is not easy to know when this happens. Another is that anyone acutely infected with these viruses may spread the infection to others in much the same manner that anyone with a cold might infect his intimate associates. Thus involuntary vaccination may result, and it is easy to see how there could be opposition on the part of the public to this idea, perhaps comparable to the kind of objection occasionally encountered about putting fluorides in the water supply.

As to the origin of these attenuated polioviruses, some of them have been produced in the laboratory, others have been picked up in the so-called "wild" state. Those which have been prepared in the laboratory have been subjected to a process of domestication as it were. The most frequently used method of domestication or of taming these strains of virus has been that of starting with a virulent strain of poliovirus and growing it continuously with multiple passages in tissue cultures. What happens under these circumstances is that the dangerous strain of poliovirus in the course of adapting itself to a new and artificial environment may lose the property of virulence. After prolonged transfer of this kind, often by means of the terminal dilution techniques, the assumption has been made that this loss of virulence becomes more or less fixed and the strain of modified virus can be considered relatively safe for use as an immunizing agent in man. Some of the tests employed to check this are measures of the neurovirulence in the monkey on both intracerebral or intraspinal inoculation; there are also a number of other measurable properties more or less related to virulence, but the ultimate tests of safety, and by far the most extensive, have been made on man. In these field trials on human populations, the objective has been to see whether there has been any evidence of illness in those who have received the vaccine, i.e., vaccines, or whether any illness traceable to infection by the attenuated poliovirus has been evident among the vaccinee's neighbors. Surveys have also been made to see whether the domesticated virus may or may not gradually work its way back to being a dangerous virus during the time in which it multiplies within the body of a single person or whether virulence becomes enhanced during natural human-to-human passage. Obviously, if reversion to virulence occurs, this would be an undesirable event. All one can say at present is that illness which has been the proven result of an infection by attenuated poliovirus given as a vaccine has to date not been noted. It has been noted nonetheless that attenuated virus excreted in stools occasionally shows evidence of having undergone some changes and even to have become slightly more

virulent than when fed. However, such viruses are still in that attenuated range where they are relatively harmless.

In the United States small field trials have been carried out over the past six or eight years, in New York State,[11] Ohio,[17] California,[16] Connecticut,[6,7] Louisiana,[8] and Arizona.[8] A larger trial has recently been reported from Minnesota.[10] Many other trials have been conducted abroad, within such countries as the Belgian Congo,[8] Uruguay,[10] Chile,[1] Colombia,[10] Nicaragua,[10] and Mexico,[10,18] In Costa Rica, a campaign which aims to vaccinate all the children under 13 years of age has been in progress during 1959.[10,21] In Europe, trials have been carried out in Holland,[20] England,[8] Finland,[10] Poland,[10] and Czechoslovakia.[10] It is certain that others not mentioned here have also been done. However, the most extensive and significant of the large-scale tests are those which have been carried out in 1959 in the Soviet Union.[10] This program now involves 11 of the 15 Soviet Republics and covers about 10,000,000 individuals. Plans are underway to enlarge these to 15,000,000 before 1960 and perhaps 60,000,000 by 1961.[9] The results so far seem to be satisfactory and among the millions of persons, including infants, children, and adults, who have received this vaccine to date there has not been a single unfavorable report.

Nonetheless, quite a few questions still remain to be answered about the live vaccine. How many individuals, particularly the children who have received the vaccine, are actually immunized as a result of this experience is a question with high priority. It appears that this measure of efficiency differs in different environments. Without laboratory tests it is impossible to determine whether a "take" has occurred or not. Failure to take can be due to several factors. The phenomenon of interference, for instance, can be one of them—interference not only by wild polioviruses but by other enteroviruses. At the present time trials are being conducted to determine whether by increasing the dosage this deficiency can be overcome.

One may ask here, with justification, why is there any need for a new poliomyelitis vaccine if the Salk-type vaccine is successful and will continue to be used and if there are so many unknowns with regard to live poliovirus vaccine? A primary answer to this is that, after all, it is logical that a search for improvements in methods of protecting against this serious disease should continue. As mentioned earlier in this article, the Salk-type vaccine has not been found to protect all those to whom it has been given in three doses. In the U.S.A. in 1959 this could amount to 10 or 15 per cent of the 5,000 or more reported paralytic cases. As the ultimate objective is to protect *all* by methods that will prove practical, a search for improvements should continue. Furthermore, the Salk-type vaccine

is still moderately expensive for some countries, notably several in Latin America and elsewhere, which might wish to use this vaccine on a large scale but have claimed that they cannot afford to do so. This may not be a valid criticism, for the cost of the live poliovirus vaccine is as yet an unknown quantity. Another criticism of the Salk-type vaccine is that the way in which it is necessary to give it—two inoculations four to six weeks apart with a third not earlier than seven months later, and a fourth a year after the third dose—is a difficult schedule for some people to follow. The lag in persuading people in the United States to take advantage of the Salk-type vaccine and to follow the proper schedule suggests that an equal or greater task might exist in those countries less attuned to the idea of prophylactic inoculations than is the United States. The live poliovirus given by mouth (three times, a month apart, for the monovalent live vaccine or perhaps *not more* than twice a month or six weeks apart for the trivalent) sounds easier administratively. It may be important to find out whether that fraction of the U. S. population which has refused or failed to take advantage of the Salk-type vaccine will also refuse or not even be interested in the live poliovirus vaccine. Thus it remains to be seen whether the live attenuated poliovirus vaccine will better the record of Salk-type vaccine. The suggestion that it might is a challenge worth accepting. As a hypothetical means of promoting the universal administration of live poliovirus vaccine it has even been suggested, and this is under trial, that following an initial and vigorous campaign in the local use of this vaccine for children and young adults it might then be given to *all* newborn babies and by this means the immunity of the entire population could consequently be kept up. It remains to be seen whether parents or hospitals would accept this responsibility or indeed whether newborn babies would be sufficiently easily infected. The chances of their having interfering enteroviruses during the first days of life would seem slim but infants may have a natural form of resistance which might be difficult to overcome in order to get a take. It is easy to see how all these important questions demand vigorous attention and extensive trials. In any event, if untoward incidents or deficiencies are to occur, it is far better to have them occur during the trial stage than afterwards when the vaccine has been approved and licensed.

In the meantime it seems unlikely that the people of the United States will quickly abandon the Salk-type vaccine in favor of a new product, particularly as the former is being steadily improved. However, during the next few years at least there may be an increasing use of a combination of the two, i.e., a course of killed vaccine followed by live vaccine. Theoretically this might yield a higher degree of protection than the use of either vaccine alone.

To summarize the present situation, we face the fact that the only recognized and official prophylactic agent against poliomyelitis in the United States is the Salk-type vaccine. Its continued and wider use should not only be encouraged here but urged. The live poliovirus vaccine should be regarded as something of considerable promise which is still in the trial stage. The present results of field trials, some of which have been very extensive, are sufficiently encouraging to indicate that they should proceed with dispatch if this promising new prophylactic measure is to have the widespread use it may ultimately deserve.

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THE USE OF THE EAR OXIMETER FOR MEASUREMENT OF THE CARDIAC OUTPUT DURING WALKING EXERCISE IN HUMAN SUBJECTS‡

INTRODUCTION

For the study of walking exercise in human subjects, an accurate method for measurement of the cardiac output is needed which can be applied repeatedly and with minimal discomfort to the subject. Continuous recording of dye-dilution curves by means of an ear oximeter has been used previously,[1, 9, 10] for estimating the cardiac output at rest, and requires only venipuncture. However, the accuracy of this method has been seriously questioned for the following principal reasons:

a. Dye-dilution curves obtained from the ear frequently differ in form from those obtained simultaneously from a systemic artery.

b. The presently available commercial¶ earpiece has been used in prior studies without full consideration of the photometric characteristics of the earpiece system.

c. Methods for the calibration of earpiece curves, in terms of mg. per cent of dye in arterial blood per cm. of galvanometer deflection, have been inadequate.

The present study was therefore undertaken with the following aims:

a. To define the photometric characteristics of the Waters earpiece cuvette, and by application of these characteristics, to improve the instrumental technique for recording Evans Blue dye curves.

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Work done during Dr. Goodyer's tenure of an Established Investigatorship of the American Heart Association, Dr. Chetrick's tenure of a Research Fellowship of the American Heart Association, and Dr. Huvos's tenure of a postdoctoral research fellowship of the U.S. Public Health Service.
¶ Waters Corporation, oximeter earpiece.
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b. To explore the validity of using a standard calibration factor for curves in different subjects, by comparing cardiac outputs estimated simultaneously by the earpiece method and by either the Fick or Hamilton (direct arterial sampling) methods.

METHODS

A. *Instrumentation studies:*

The performance of a modified Waters earpiece in accordance with the theoretic relationships described in the *Appendix* was explored. A slit-like tunnel overlying the earpiece photocell was constructed, using sections of clear plastic microscope coverslips affixed to the earpiece by means of suitable liquid cement. Gelatin filter-wedges could then be inserted into the tunnel either when the earpiece was on a subject's ear, or when one of the filters ordinarily supplied with the earpiece ("E90" or "E35") replaced the ear. Color filters having absorption curves identical to that of Evans Blue, and of sufficiently low density to fall in the same range as encountered with actual dye curves, were not available. Filters were therefore selected which had low densities for the red cell and essentially zero density for the infrared cell (Kodak CC20R and CC20M). Other filters (ND0, ND10, and CCB05), which affect both infrared and red cells, were used to vary initial lightpath conditions and to study galvanometer responses to colors affecting both the photocells.

B. *Application of instrumental techniques*

The formulae given in the *Appendix* and the results of the above instrumentation studies indicated that it should be possible to estimate accurately the cardiac output from a dye curve recorded with the earpiece, under the following assumptions:

a. That the approximately linear relation (see *Results*) between galvanometric deflection and dye concentration holds true for blood distributed in the ear tissues, and can be determined for each earpiece system;

b. That the "primary" areas (determined by logarithmic replot) of curves of dye concentration in the ear vessels are directly proportional to the corresponding areas of simultaneous curves in the major arteries.

These assumptions were explored in the present study by comparing values of cardiac output determined from dye curves obtained simultaneously by the earpiece technique and by direct arterial sampling, or by the Fick method. Twenty-one ambulatory subjects who were free of clinical cardiopulmonary disease, were studied at rest and in some cases during the fourth minute of exercise on a motor-driven treadmill set at a grade of 7 degrees and at a speed of 1.2 to 3.5 mph, depending on each subject's tolerance for exercise as determined during a pre-study trial. Dye curves were produced by the injection of exactly 2 cc. of a 0.5 per cent solution of Evans Blue dye into the central circulation through a 70 cm. length of polyethylene tubing (PE 50) which was inserted through an 18-gauge needle into an antecubital vein, the needle being then withdrawn around the catheter. The position of the catheter tip within the right atrium was verified by visualization, through an image intensifier, of radio-opaque material introduced temporarily into the catheter. An alternate method of injection utilized a 6-inch segment of polyethylene tubing containing 2 cc. of 0.5 per cent dye, connected at one end to a stopcock and short cannula inserted into the antecubital vein, and at the other end to a stopcock and saline-filled syringe. Ten cc. of saline was used to flush

rapidly (in 1-2 seconds) the dye-column into the venous system. Earpiece curves were recorded at least ½ hour after vasodilation had been induced in the pinna by application of a local vasodilator and by the warmth of the earpiece light-source. Arterial sampling was accomplished by fractional collection (in a Newman type rotating collector[7]) of blood flowing freely at 2 cc./sec. from the femoral artery via an 18-gauge, thin-walled needle and a 50 cm. length of polyethylene (PE #320) tubing. The centrifuged fractional specimens were analyzed for plasma dye content in terms of optical density units by colorimetric comparison at 620 mμ against a dye-free plasma blank. An exact measurement of the amount of dye injected was determined separately for each experiment by injecting a duplicate bolus of dye, exactly as described for the subject, into a 1-liter flask which was filled to the mark with saline. The amount of dye in the flask was then determined in terms of optical density units. The blood hematocrit was separately determined. The cardiac output was calculated from each logarithmically replotted arterial dye curve according to the standard formula:

$$\dot{Q}_A = \frac{I \times 60}{\Sigma(\text{O.D.'s})} \times \frac{1}{1\text{-Hkt}}, \text{ where}$$

$\dot{Q}_A$ = Cardiac output, liters/min.

I = Amount of dye injected, expressed in optical density units (O.D.'s)

Σ(O.D.'s) = Sum of dye concentrations in plasma at each second of the primary earpiece curve, expressed in optical density unit-seconds/liter

Hkt = Blood hematocrit

Cardiac output was estimated by the Fick method simultaneously with the earpiece technique 13 times at rest and 10 times during walking exercise. For this purpose, central venous oxygen content was determined in blood withdrawn from the right auricle through the catheter used for central injections of dye as described above. Arterial oxygen content was taken as 95 per cent of the determined oxygen capacity, which is a normal value for this laboratory and the average corrected value for direct oximetric estimations of arterial oxygen content under the conditions of these experiments.

Oxygen consumption was calculated from the volume and oxygen content (Scholander method[8]) of expired gas collected in a Douglas bag through a low-resistance (Rudolph) valve from the subject breathing room air.

The calibration factors (S_D) for calculating the areas of the earpiece curves were derived as follows:

a. From the arterial dye curves:

$$S_D = \frac{\Sigma(\text{O.D.'s})\,(1\text{-Hkt})}{\Sigma(Y)}$$

where: Σ(O.D.'s) and Hkt are as defined above

$\Sigma(Y)$ = sum of the galvanometer deflections at each second of the logarithmically replotted earpiece curves, expressed in millimeter-seconds

S_D = calibration factor, expressed as optical density units per liter of blood per millimeter of galvanometer deflection

b. From the Fick values for cardiac output ($\dot{Q}_A$, Fick expressed in liters per minute):

$$S_D = \frac{60I}{\Sigma\,(Y)\;(\dot{Q}_A, \text{Fick})}$$

These calibration factors were based on the entire areas of the logarithmically replotted oximetric curves rather than on the peaks or on other selected points of the curves. They therefore allowed for the variably slurred contours of some earpiece dye curves (see *Results*).

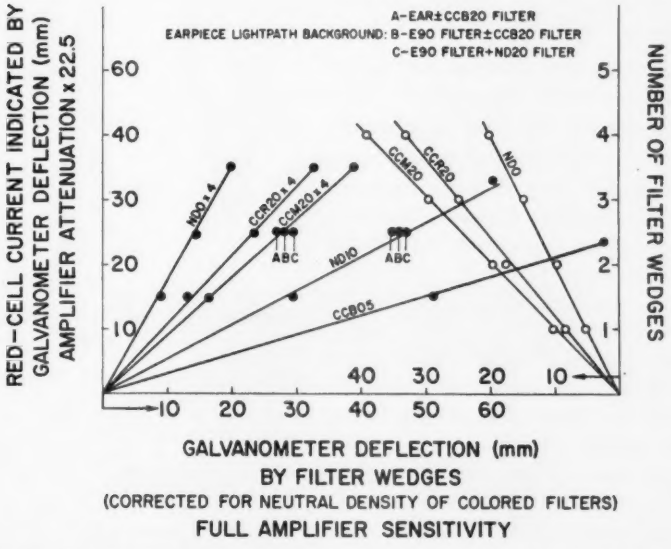

FIG. 1a. Galvanometer deflections produced by different filter wedges (defined in text) at various measured levels of amplifier input current from the red photo-cell (left-hand scale) and using various multiples of each filter wedge (right-hand scale). Changes of earpiece lightpath background by three combinations of ears, E90, CCB20, and ND20 filters (A, B, C) caused slight variations of the galvanometric deflections by the CCM 20 and ND10 filter wedges at the same input current settings (dots labeled A, B, C).

RESULTS

1. The results of the studies of the photometric characteristics of the Waters earpiece system are summarized in Figures 1a and 1b. The "square wave" response of the earpiece-amplifier-galvanometer system to a sudden change of color density is illustrated in Figure 1a. This response characteristic was similar to those of other indicator dilution recording methods,[4] and was considered adequate for faithful recording of dye-dilution curves.

Two of the basic theoretic relationships (formulae 6 and 7) were confirmed, as indicated in Figure 1b which shows:

a. The approximately linear influence of the initial photocell current on the galvanometric deflection caused by a given filter wedge.

b. The approximate linearity of the relation between color density and galvanometric deflection, when the initial photocell current is constant, irrespective of the initial lightpath conditions.

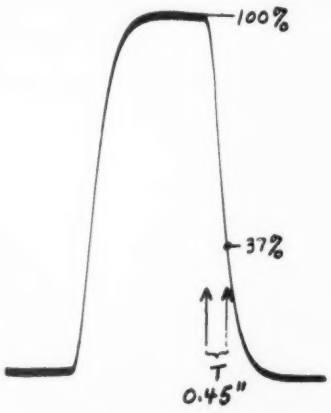

FIG. 1b. Characteristic response of earpiece-amplifier-galvanometer system, used in the present studies, to a rapid change of photocell illumination produced by suddenly introducing a standard filter wedge into the earpiece lightpath and then suddenly withdrawing it. The time for fall of the galvanometer beam to 37 per cent of its total deflection (the *time constant*) was 0.45 seconds.

2. Forty-nine dye dilution curves were recorded by the earpiece method for the present study. Specially *selected earpiece curves* are shown in Figures 2a, b, and 3 where they are compared with simultaneous dye curves obtained in normal subjects by direct fractional collection from the femoral artery, and with a simultaneous thermal dilution curve recorded by intra-aortic thermistor in a patient with mitral stenosis and regurgitation during left heart catheterization according to a recently developed method.[4] The close similarities of timing and contour of these paired curves are evident. However, 28 of the 49 earpiece curves had more prolonged downstrokes and less prominent recirculation peaks than were observed in simultaneous curves obtained by direct fractional collection of arterial blood. In 10 of these curves, recirculatory inflections were absent or occurred so high on the downslopes of the primary curves that the latter could not be defined adequately by logarithmic replots. It was therefore necessary to establish criteria for the rejection of earpiece curves with characteristics significantly different from those expected or observed in simultaneous curves sampled directly from an artery. On the basis of the range of normal variations found in the directly sampled arterial curves of this study, the following arbitrary criteria indicating a "satisfactory" earpiece curve were defined:

a. Absence of major irregularities of the curve or of its preliminary baseline.

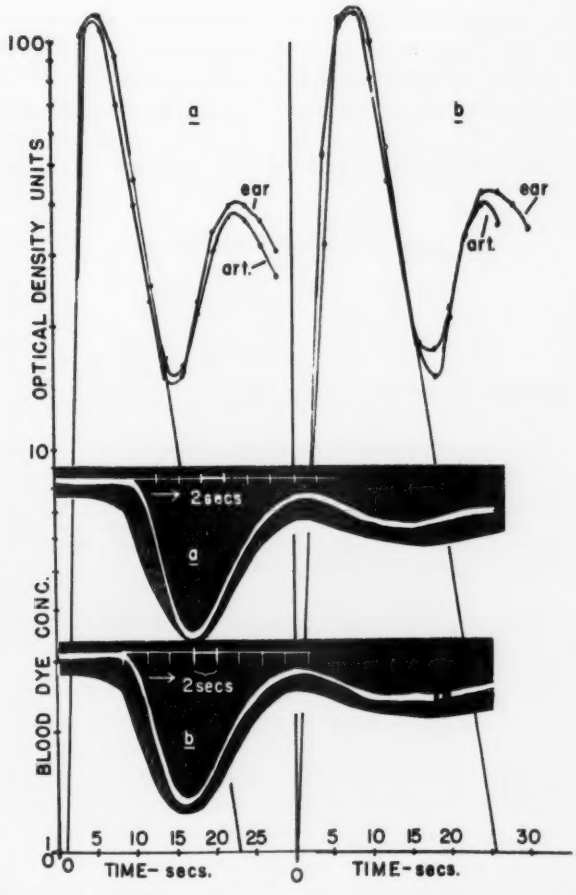

FIG. 2. Semi-logarithmic plots of dye dilution curves obtained simultaneously by continuously recorded earpiece colorimetry and by direct analyses of successive samples of arterial blood collected fractionally. The original earpiece curves are shown in the insets.

b. Primary peak more than 20 mm. (equivalent to an arterial dye concentration more than 6 mg./l).

c. Disappearance slope* of the log replot clearly defined as a straight line for at least four seconds, and having a numerical value larger than *0.14* (the minimum slope found in the directly sampled arterial curves).

In accordance with these criteria, 18 earpiece curves of the present study were rejected, although it was recognized that a few valid curves with low peaks during exercise may thus have been excluded. The disappearance

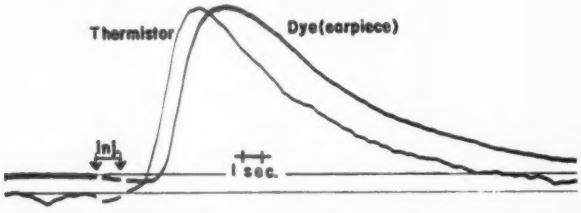

FIG. 3. Earpiece dye and aortic thermal dilution curves recorded simultaneously during left heart catheterization of a patient with mitral stenosis and regurgitation. Five cc. bolus of dyed saline introduced via the left auricular needle at the arrows. Recirculation peaks missing (normally) in thermal curve, very low and delayed in dye curve, due in part to cardiac disease. This dye curve would have been rejected for the present study, if obtained in a patient without heart disease, because of the slow descending slope and minimal recirculation peak.

slopes of the remaining 31 curves averaged —0.29, with a range of —0.14 to —0.58.

3. Standard calibration factors (see *Methods)* for expressing the areas of earpiece dye curves in terms of optical-density-unit-seconds per liter were determined for each of two earpiece units by equating the averaged cardiac output values determined with each earpiece $(\dot{Q}_A)_2$, Fig. 4) with the averaged values simultaneously determined by the Fick or arterial-sampling methods $(\dot{Q}_A)_1$, Fig. 4). Variations of all individual values of the ratio $(\dot{Q}_A)_2/(\dot{Q}_A)_1$ are indicated by the scatter of points in Figure 4 between the ranges of about *1.33* and *0.67*, with a standard deviation of ±0.18. In 18 experiments, the change of this ratio, $(\dot{Q}_A)_2/(\dot{Q}_A)_1$, was studied when, without disturbing the earpiece placement, measure-

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* Disappearance slope $= \dfrac{\log_e C_2 - \log_e C_1}{t_2 - t_1}$

where: C_1 and C_2 are points chosen at random on the disappearance slope before the recirculatory inflection;
t_1 and t_2 are the corresponding times, in seconds.

ments were repeated in the same subject at rest (9 experiments) or during exercise (9 experiments). Under these conditions, experimental errors involved in correcting (by formula 13) the standard calibration factor used for determining $\dot{Q}_{A1}$, were eliminated. The ratio, $(\dot{Q}_A)_2/(\dot{Q}_A)_1$, changed less than ± 13 per cent in all cases. Since this figure included the errors of both earpiece and comparative methods, the earpiece method had an

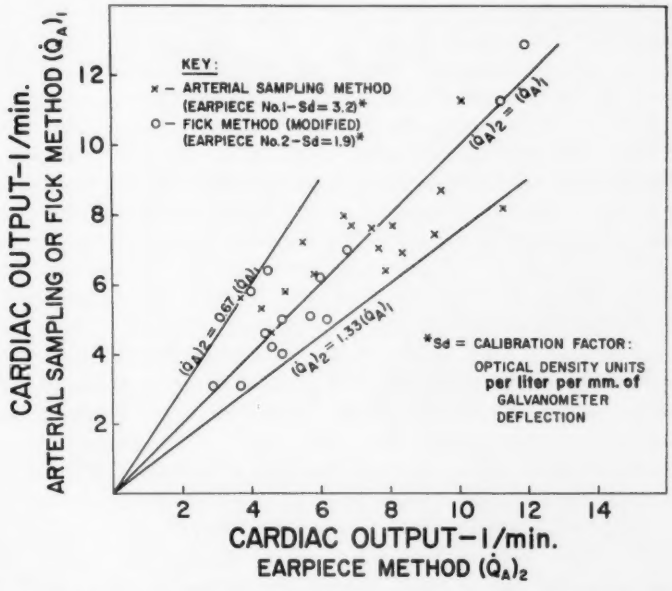

FIG. 4. Variations of cardiac output determinations by the earpiece colorimetric method from those obtained simultaneously by the Fick and the arterial sampling methods, using single calibration factors (Sd) for each of two earpieces.

observed error of *less than ±13 per cent* when used to determine the proportionate change of cardiac output in the same resting or exercising subject, provided the placement of the earpiece was unchanged.

DISCUSSION

The results of the present study indicate that photometric characteristics of the Waters earpiece cuvette system are adequate for essentially linear recording of small changes of optical density of the earpiece light-path. Discrepancies between the contours of earpiece dye-curves and simultane-

ous arterial dye curves must be attributed, therefore, to inadequacy of the ear pinna as a system for rapid and continuous sampling of dye-color in arterial blood. The probable cause of such inadequacy may be surmised from prior analyses of mechanical sampling systems[3, 5] and from theoretic considerations of the indicator-dilution method itself:[6] the central arterial dye curve is continuously sampled by the auricular artery and then by many parallel nonuniform vascular circuits contained in the earpiece light-path, each of which has a transit time for dye particles which varies with the character and velocity of local blood flow. At any one instant, therefore, the earpiece photocells "sense" an appreciable time-integral of the curve of dye-concentration in the peripheral artery supplying the ear pinna. Rapid flow of blood through the ear vessels would, logically, tend to minimize this "smearing" of the arterial curve by satisfying the theoretic requirement of a more temporally punctate and uniform sampling site,[6] as well as the empiric requirement of a high blood flow in relation to total volume of the sampling system.[5] That these requirements can be satisfied by the earpiece-pinna system, when the vessels of the ear are widely dilated, is indicated by the almost identical contours of simultaneous earpiece and arterial curves such as those of Figures 2 and 3. Since many earpiece curves are not as ideal as these, the validity of the earpiece method for estimating cardiac output depends greatly on the exclusion of curves which are not adequately representative of those expected or simultaneously observed in a major artery. The criteria used in the present study for defining acceptable curves, based on the range of variations of the directly sampled arterial curves, were necessarily arbitrary. Nevertheless, the minimum acceptable numerical value of the disappearance slope (0.14) was within two standard deviations (0.11) of the normal mean value (0.24) defined in another report for 9 radial artery curves in adult men.[12] The empiric validity of these chosen criteria was affirmed by the fairly close correlations (Fig. 4) of values of cardiac output determined simultaneously from selected earpiece curves and from measurements using either the arterial-sampling or Fick methods.

Application of the earpiece technique to the study of exercise requires consideration of several limitations of the earpiece method defined in the present study:

a. Determination of absolute values of the cardiac output in different individuals, using a standard calibration factor as described above, entails an error of about ± 33 per cent (Fig. 4). A smaller error of about ± 13 per cent is involved in comparisons of values of cardiac output determined in the same individual without changing the earpiece placement. For the

study of exercise, such errors may be allowed, since changes of cardiac output of the order of 200-300 per cent, or more, may be expected.

b. The arbitrary criteria used to designate "satisfactory" curves may be expected to result in the rejection of about 35 per cent of all recorded earpiece curves (see *Results*). Furthermore, this loss of data may be expected to increase if patients with enlarged hearts are studied, since an increased "central" blood volume may seriously distort the arterial dye curve itself. In such circumstances, it may be necessary to validate at least one earpiece curve of each subject by direct comparison with a simultaneous arterially sampled indicator dilution curve.

SUMMARY

1. The photometric characteristics of the Waters earpiece cuvette, which are pertinent to the quantitative registration of Evans Blue dye curves, were defined.

2. Using arbitrary criteria for the selection of "satisfactory" earpiece curves, and a standard calibration factor for each of two earpieces, close correlation was demonstrated between values of cardiac output estimated simultaneously by the earpiece method and by either the Fick or Hamilton (direct arterial sampling) methods.

3. The earpiece method appears to be well suited to the determination of cardiac output in exercising human subjects.

ACKNOWLEDGMENT

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APPENDIX

Theoretic considerations of the photometric characteristics of the Waters earpiece cuvette and amplifier system used in the present studies.

The Waters earpiece cuvette consists of two sets of photocells facing a polychromatic light source, and designated "red" and "infrared" to indicate peak transmittances at 630 mμ and 800 mμ, respectively. If Beer's law is approximately applicable to this system, and the currents of the photocells are proportional to the light transmitted through the earpiece light-path, these currents will change during the passage of a dye-solution through the light-path, according to the following relations:

$$I_R = I_R^0 \cdot e^{-wd_1} \text{ and } I_{IR} = I_{IR}^0 \cdot e^{-wd_2} \quad (1)$$

where: I_R^0 and I_{IR}^0 are the red and infrared photocell currents before the passage of dye,

I_R and I_{IR} are the photocell currents during passage of dye,

d_1 and d_2 are the characteristic optical densities at 630 mμ and 800 mμ, resp., of the layer of dye interposed in this earpiece light-path system,

$w = 1/\log_{10} e = 2.3$

In the present studies, the currents of the red and infrared cells were applied to a differential amplifier,** the output of which drove an oscillograph*** galvanometer, the deflection of which was proportional to the difference between selected fractions of the full photocell currents. That is,

$$G_R = A_1 K_1 I_R;\ G_{IR} = A_1 K_2 I_{IR} \quad (2)$$

and

$$\pm G = G_{IR} - G_R = A_1 (K_2 I_{IR} - K_1 I_R) \quad (3)$$

where: $K_1 I_R$ and $K_2 I_{IR}$ are the fractions of the full photocell currents selected by potentiometers of the amplifier input circuit, so as to bring the galvonometer to an arbitrary "zero" position, where $K_1 I_R^0 = K_2 I_{IR}^0$

G is the net galvanometer deflection, resulting from opposition of the selected photocell currents. The sign of G (+ or —) depends

** Electronics for Medicine, Model ODC-8sc.
*** Hathaway, S14A Student Oscillograph.

on the polarity of the amplifier input or output connections and is arbitrary.*

G_R and G_{IR} are the galvanometer deflections which would be produced by the selected currents from the red and infrared photocells, respectively, if these currents were not opposed.

A_1 is the amplifier factor, expressed as mm. galvanometer deflection per unit of unbalanced input current.

Since Evans Blue dye scarcely affects the infrared cell ($\Delta K_2 I_{1R} = 0$), the galvanometer deflection caused by this dye will vary with the change of current of the red cell ($G = -\Delta G_R$).

From (1) and (2):

$$G_R \text{ (during the dye curve)} = A_1 K_1 I_R^0 e^{-wd_1} \tag{4}$$

By differentiation:

$$\frac{dG_R}{d(d_1)} = -w A_1 K_1 I_R^0 e^{-wd_1} \tag{5}$$

which approaches $-w A_1 K_1 I_R^0$ for small values of d_1 ($\Delta d_1 (= d_1) \to 0$) such as apply to concentrations of dye ordinarily encountered in dye-dilution curves *in vivo*.

Therefore:

$$-\Delta G_R = G \simeq w A_1 K_1 I_R^0 \Delta d_1 \simeq w A_1 K_1 I_R^0 d_1 \tag{6}$$

and:

$$\frac{Ga}{Gb} \simeq \frac{d_1^a}{d_1^b} \times \frac{(K_1 I_R^0)_a}{(K_1 I_R^0)_b}, \text{ if } A_1 \text{ is constant} \tag{7a}$$

$$\frac{Ga}{Gb} \simeq \frac{d_1^a}{d_1^b}, \text{ if } A_1 \text{ and } K_1 I_R^0 \text{ are constant} \tag{7b}$$

$$\frac{Ga}{Gb} \simeq \frac{Ca}{Cb}, \text{ since } \frac{d_1^a}{d_1^b} = \frac{Ca}{Cb} \tag{8}$$

where:

Ga and Gb are the galvanometer deflections induced by equal thicknesses of dye solution of densities d_1^a and d_1^b corresponding to dye concentrations of C^a and C^b, respectively.

* In the present studies, dye curves were at first recorded so as to indicate the passage of dye by a negative galvanometer deflection (as illustrated in Fig. 2). Formulae (4) et seq., and the discussion of dye curves in the text, conform to the present convention of indicating the pasage of dye by a positive galvanometer deflection.

$(K_1 I_R^0)_a$ and $(K_1 I_R^0)_b$ are the initial values of the selected photocell currents before the passage of dye in each case.

The approximately linear relation between the galvanometer deflection per unit change of dye density (G/d_1) and the initial ("pre-dye") photocell current (see formula 6 above) suggests that earpiece dye curves in different subjects might be calibrated by a single factor (C/G, the change of blood dye concentration represented by a 1-mm. galvanometer deflection) if the initial red cell current presented to the amplifier ($K_1 I_R^0$) were adjusted to a constant value (formulae 6-8). However, the change of red cell current induced by a unit change of blood dye concentration also depends on the thickness of blood in the earpiece light-path, since (by definition)

$$d_1 = \beta_1 C l \tag{9}$$

where: C = concentration of blood dye

l = thickness of blood layer

β_1 = optical density of a layer of blood of unit thickness and unit dye concentration. This absorption coefficient is specific (and different) for each earpiece unit.

It is, therefore, necessary, in using the earpiece on different ears or when the placement of the earpiece is changed, to correct any standard calibration factor, C/G, for individual variations of d_1 due to changes of the thickness of the blood layer in the earpiece lightpath. These variations may be estimated according to the following considerations:

Let: I_R^B = the red cell current when the ear is perfused with blood.

I_R^T = the red cell current when blood is pressed from the ear by inflation of a rubber diaphragm in the earpiece light-path to a pressure above arterial.

h = the optical density (at 640 mμ) of the layer of oxygenated blood interposed in the earpiece light-path.

H = the blood hemoglobin concentration.

l = the thickness of the blood layer in the earpiece light-path.

β_2 = the optical density of a layer of blood of unit thickness and unit hemoglobin concentration (specific for each earpiece unit).

If Beer's law holds:

$$K_1 I_R^B = K_1 I_R^T e^{-wh} \tag{10}$$

and $$wh = \log_e K_1 I_R^T - \log_e K_1 I_R^B \tag{10a}$$

Also,

$h = \beta_2 H l$, as defined above

and, from (9), and l may be obtained from (9) to give

$$h = \frac{\beta_2 H d_1}{\beta_1 C} \tag{11}$$

If the thickness of the layer of blood changes from l to l', and if the blood hemoglobin concentration changes from H to H', d_1 for a given blood dye concentration (Ca) will change to some value d_1', such that (from formula 11)

$$\frac{l'}{l} = \frac{d_1'}{d_1} = \frac{h'\,H}{h\,H'} \tag{12}$$

and, from formulae (4), (6), and (7) substituting $K_1 I_R^B$ and $K_1 I_R^{B'}$ for $K_1 I_R^0$ in formula (4),

$$\frac{Ga'}{Ga} \sim \frac{d_1'(K_1 I_R^{B'})}{d_1 (K_1 I_R^B)} \sim \frac{h'\,H\,(K_1 I_R^{B'})}{h\,H'(K_1 I_R^B)} \tag{13}$$

where: *Ga'* and *Ga* are the galvanometer deflections induced by the blood dye concentration, Ca, but corresponding to changes of l and H to l' and H', respectively.

$K_1 I_R^{B'}$ and $K_1 I_R^B$ are the selected initial red photocell currents corresponding to Ga' and Ga, respectively.

Formula (13) is simplified if $K_1 I_R^{B'}$ is adjusted to the same constant value, $K_1 I_R^B = X$, for all "heat-flushed" ears. h'/h may then be determined for each earpiece placement by using formula (10) as follows:

$$\frac{h'}{h} = \frac{\log_e(K_1 I_R^{T'}) - \log_e X}{\log_e(K_1 I_R^T) - \log_e X} \tag{14}$$

where: $K_1 I_p^T$ and h are the average values for a group of representative earpiece placements.

$K_1 I_p^{T'}$ and h' are the measured or derived values for each earpiece placement.

$K_1 I_p^{T'}$, $K_1 I_p^T$, and $X = K_1 I_p^B$, may be measured directly by noting the galvanometer deflections induced by unopposed red cell currents at an attenuation about 25 times greater than that used for recording dye curves.

By definition, *Sd* (the standard calibration factor obtained as described under *"Methods"*) is defined by: $Sd = C/G$ (i.e., the change of blood dye concentration represented by a 1-mm. galvanometer deflection). A corrected calibration factor (*Sd'*) may, therefore, be obtained from formula (13), since:

$$\frac{Ga'}{Ga} = \frac{Sd}{Sd'}, \text{ where } Ca = C,$$

If $K_1 I_R^{B'}$ and $K_1 I_R^B$ are always set to the same value, (X), for all measurements, including those used for establishing Sd (see *Methods*),

$$\frac{Sd}{Sd'} = \frac{h'H}{h\,H'}$$

If $K_1 I_R^{B'}$ is different from X in any instance, it may be measured directly for use in Formula (13). However, an alternate method is available for determining the ratio $K_1 I_R^{B'}/K_1 I_R^{B}$: The galvanometer deflection produced by the insertion into the earpiece light-path of a standard filter wedge (see Methods) will vary according to the ratio $K_1 I_R^{B'}/K_1 I_R^{B}$ (Formula 13), and will also reflect changes of the over-all earpiece amplifier system due to latent changes of β_1, the dye-absorption coefficient specific for each earpiece unit, and of A_1, the amplifier factor, (Formulae 6 and 9).

Using this method,

$$\frac{Sd}{Sd'} = \frac{h'\,H\;G_w'}{h\;H'\,G_w}$$

where:

G_w is the galvanometer deflection produced by the standard filter wedge when $K_1 I_R^B$ was set at X during the experimental determination of Sd (see *Methods*).

G_w' is the observed galvanometer deflection produced by the standard filter wedge.

Some of the formulae given above are similar to those derived elsewhere for a different instrumental system (9).

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THE EFFECT OF LANATOSIDE-C ON THE RESPONSE OF THE HUMAN CARDIAC OUTPUT TO WALKING EXERCISE

When the possibility of heart failure is anticipated in the human subject during exercise, or during some other form of circulatory stress such as a surgical operation, the chance of benefit from the administration of digitalis must be weighed against the probability of circulatory impairment by the drug if no heart failure appears—a probability which has been suggested by prior clinical observations,[28] and by physiological studies in resting human subjects free of heart failure.[4, 11, 16, 20] However, the changes of cardiac output caused by digitalis at rest may not necessarily indicate what effects the drug will have on the stressed circulation. In the normal dog, for instance, the resting systemic blood flow is reduced[7] by a dose of cardiac glycoside which increases ventricular contractility,[5] and which improves the output of the heart working under an increased pressure or flow load.[25] It is apparent that the effect of digitalis on circulatory competence is best measured by a change of cardiac output when the heart is operating near the limit of its capacity, or when the adequacy of the output may be expressed in terms of concomitant peripheral circulatory demands.

The present investigation was prompted by the need for more definitive information regarding the effect of cardiac glycosides on the normal human circulation under stress. With the aforementioned considerations in mind, it was proposed to evaluate the effect of Lanatoside-C on the circulatory response to some form of exercise commonly required of the human subject. For this purpose, it was of paramount importance to choose the proper *type* and *degree* of exercise with which to challenge the circulation, as well as the most suitable measurements by which to evaluate the circulatory response.

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The types of exercise, i.e., *bicycling* and *supine pedaling* which have been used for most pertinent previous studies of the circulation during muscular effort,[2, 6, 9, 15, 23, 27] cause changes of heart size, rate, and output which are quite different from those elicited by *walking,*[1, 28] which was chosen for the present studies because it is a form of exercise familiar to all subjects, for which no special training is needed and to which, therefore, more reproducible circulatory responses during repeated studies may be expected.[28]

The degree of exercise necessary to detect cardiac impairment depends not only on the extent of this impairment but also on the method used for its detection. Although *exhausting exercise* is needed to define the respiratory limits of the physiological response to effort,[21, 30] *"submaximal exercise"*[2] may be used to define circulatory deficiences of the response[9, 10, 20] and was chosen for the present studies because it was more reasonably applicable to ordinary hospital patients.

The most reliable and sensitive parameters for evaluating the circulatory response to exercise in human subjects include tissue anaerobiasis,[19, 20] regional redistribution of peripheral blood flow,[8] and intracardiac pressures (under special circumstances).[18] However, measurements of these parameters involve difficult and uncomfortable procedures which are not readily applicable to walking exercise or to repeated evaluations of the same subject. The *cardiac output* was chosen for evaluating the circulatory response to exercise in the present studies because of its demonstrated utility for this purpose[10, 22, 28] and particularly because of the availability of a readily applicable and reliable method for its measurement during walking exercise in human subjects.[14]

The only prior study of the effect of digoxin on the cardiac output responses of normal subjects to exercise[31] yielded results which were not without question. Digoxin appeared to diminish slightly the rise of cardiac output in six normal subjects with exercise. However, only mild supine exercise was used, and the reproducibility of the normal response of the cardiac output was not documented, so that release of anxiety[25] and training effects[1, 12] may have contributed to the tendency for the cardiac outputs during exercise after digoxin to be less than the pre-digitalization values in four of six subjects. The present study was, therefore, also designed to define the reproducibility of the circulatory response to a standard exercise in the same subject, and to define the "adequacy" of the circulatory response to exercise after digitalization by comparison with immediately antecedent studies in each subject.

METHODS

Thirty subjects, who had no clinical evidence of cardiac or respiratory impairment, were studied at rest and during the fourth minute of exercise on a motor-driven treadmill set at a grade of seven degrees and at a speed of 1.2 to 3.5 miles per hour. Each subject was given experience in walking on the treadmill during preliminary trials during which a belt-speed was selected which caused slight dyspnea, but which was easily tolerated for at least five minutes. The cardiac output was measured by an indicator-dilution method, using an earpiece cuvette, as described in detail in a separate report.[34] Ratios of the values of cardiac output ($\dot{Q}_{A_2}/\dot{Q}_{A_1}$), determined at rest or during exercise at one-hour intervals, were derived by direct comparisons of the areas of dye curves recorded from each subject with a fixed earpiece placement and after repeated injections of identical amounts of dye-indicator. In 12 studies (Table 1), Lanatoside-C was administered intravenously in doses of 1.2-1.4 mg. after the first exercise period.

RESULTS

Variations of the repeated determinations of cardiac output in both resting and exercising subjects were observed within the limits indicated in Figure 1. The values of the ratios, $\dot{Q}_{A_2}/\dot{Q}_{A_1}$, ranged approximately between 0.75 and 1.35 both for the control subjects as well as for those subjects given Lanatoside-C. No significant effect of the glycoside on the resting cardiac output, or on the normal response of the cardiac output to a standard exercise, was evident (cf. P-values, Fig. 1).

DISCUSSION

In the subjects of the present study, Lanatoside-C did not measurably alter the "normal" response of the cardiac output to exercise. However, several restrictions must be placed on the interpretation of these results:

1. The control of the cardiac output, particularly during exercise in the intact subject, is quite complicated, involving humoral, neurovascular, and peripheral circulatory factors which have no necessary relation to changes of myocardial function.[15, 24] Lanatoside-C might have altered several factors in such conflicting directions as to leave the "net" cardiac output response the same.

2. A higher dose of Lanatoside-C or more exhausting exercise might have brought out an effect of the glycoside not evident in the present studies. However, frank congestive failure is usually partly or wholly relieved by the dose of drug used herein. Furthermore, the degree of exercise used was representative of the more strenuous activity encountered by many of the present subjects in their ordinary lives. Most of the subjects would not easily have accepted more exhausting effort.

TABLE 1. CARDIAC OUTPUT ($\dot{Q}_A$) AT REST AND DURING EXERCISE. NORMAL SUBJECTS WITHOUT MEDICATION, OR ONE HOUR AFTER LANATOSIDE-C GIVEN INTRAVENOUSLY

*Expt.**					*Control measurement* $\dot{Q}_A$ - *L/min.*		*Repeated measurement* $\dot{Q}_A$ - *L/min.*	
No.	*Age*	*Sex*	*Surface area sq. meters*	*Glycoside I.V. dose mg.*	*Rest*	*Exercise*	*Rest*	*Exercise*
1	17	F	1.8	0	6.8	11.9		13.6
2	29	M	1.8	0	4.0	10.7		10.8
								12.4**
3	20	M	1.7	0	7.9	12.8		9.5
4	40	M	1.8	0	5.4	9.2		8.3
5	43	M	2.0	0	5.0	9.6		8.3
6	36	M	2.0	0	5.4	11.0		10.1
								9.5**
7	50	M	1.7	0	6.6	12.3		11.8
8	27	F	1.6	0	5.8	11.1		10.8
9	35	F	1.8	0	5.7		6.0	
10	40	F	1.5	0	3.7		2.9	
11	32	F	1.8	0	8.1		6.9	
12	30	M	1.4	0	5.5		7.7	
13	19	M	1.8	0	6.7		7.5	
14	50	M	1.4	0	4.3		4.5	
15	42	F	1.2	0	3.7		5.0	
16	35	M	2.2	0	10.1		9.5	
17	38	F	2.0	0	11.3		9.3	
18	46	M	1.9	0	8.4		7.9	
19	36	F	1.6	1.2	3.8	7.9	5.6	9.0
20	69	M	1.7	1.4	7.0	11.6	7.8	9.3
21	49	M	1.8	1.4	6.3	9.6	6.4	
22	27	M	2.1	1.4	5.8	13.6	5.8	13.1
23	19	M	1.7	1.4	4.4	8.9	5.4	8.0
24	51	M	1.8	1.4	7.3	16.6		16.2
25	70	M	1.7	1.4	4.9	9.0	5.5	8.2
26	37	F	1.5	1.4	4.7	6.8		8.1
27	40	F	1.8	1.4	3.2	5.6		7.8
28*	51	M	1.8	1.4	4.6	7.1		8.8
29*	66	M	2.3	1.4	2.6	5.6	3.1	5.6
30	49	M	1.7	1.4	6.3	12.1	5.2	9.2
								9.3**

* Subject No. 28 had a resting blood pressure of 160/95 without cardio-respiratory symptoms or cardiac enlargement by x-ray examination or ECG.

Subject No. 29 had mild pulmonary emphysema without related symptoms. All other subjects considered entirely free of cardiac or respiratory disease.

** Exercise repeated twice in subjects Nos. 2, 6, and 30.

3. The range of normal variations of the response of the cardiac output to exercise may have obscured a slight effect of Lanatoside-C. Since both physiological variation and methodologic error (less than about 13 per cent by the present method[14]) were responsible for the observed normal incon-

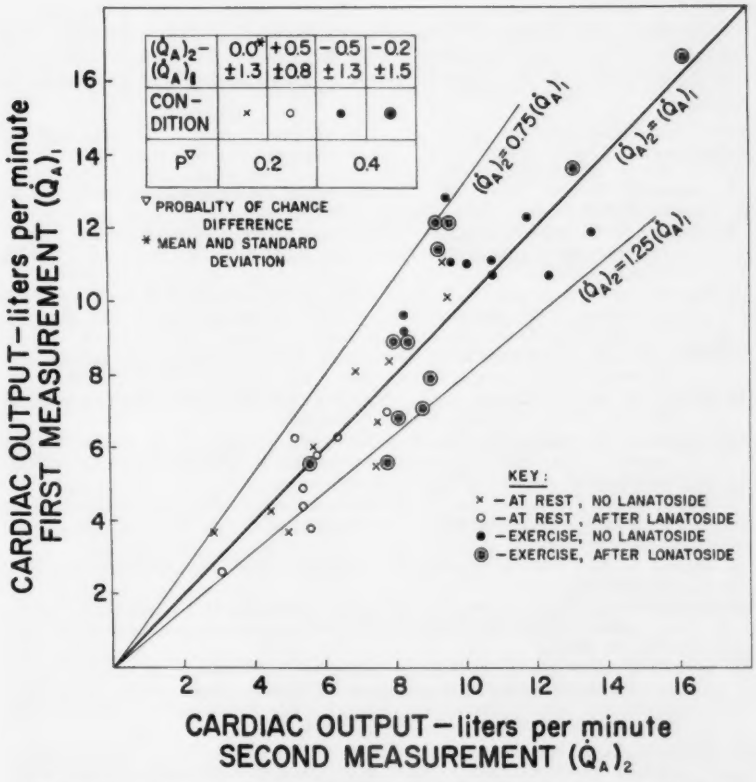

FIG. 1. Comparisons of consecutive determinations of cardiac output one hour apart in the same subject and at the same level of walking exercise, with and without the administration of Lanatoside-C.

stancy of the exercise response, any slight effect of Lanatoside-C which was undetected in the present experiments would require an entirely different approach for its elucidation. A study of the relation of the cardiac output to "anaerobic metabolism" during exercise[19, 20] might be more revealing in this regard.

With the above reservations, it may be concluded that, on the basis of the present studies, there would seem to be no valid reason for withholding a cardiac glycoside in clinical practice for fear of impairing the circulatory response to a stress comparable to moderate walking exercise.

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EXPERIMENTAL PYELONEPHRITIS

VI. OBSERVATIONS ON SUSCEPTIBILITY OF THE RABBIT KIDNEY TO INFECTION BY A VIRULENT STRAIN OF STAPHYLOCOCCUS AUREUS**

For many years it has been known that the intravenous injection of large numbers of virulent staphylococci into normal mice or rabbits regularly results in abscess formation in the kidneys.[5-7] These infections occur also in the rat.[6] Gram-negative bacilli of the coliform group, on the other hand, very rarely produce such infections when similarly administered to normal animals;[10,11] some form of renal injury is usually required for the establishment of experimental pyelonephritis by that group of bacteria. It seemed likely, therefore, that there might be significant differences in the pyelonephritis caused by these two kinds of microorganisms. The present article is a report of experiments designed to compare the course of staphylococcal infection in the rabbit kidney with that previously noted in experiments with coliform bacterial infections.

MATERIALS AND METHODS

Except where specified, the materials and methods were the same as those described in preceding reports from this laboratory.[4,9,12] All experiments were carried out in white New Zealand male rabbits weighing between 2 and 3 kilograms.

Staphylococcus aureus. This organism, known as Giorgio, was supplied to us by Dr. David Rogers of Vanderbilt Medical School. It had originally been isolated from a staphylococcal abscess in man. It is hemolytic and coagulase positive, and has been characterized in some detail by Smith and Dubos.[13] Intravenous injection of 10^7 organisms (18-hr. culture) into mice was found to cause the death of approximately two-thirds within 7 days. The organism was maintained at room temperature on agar slants. In the beginning of this study 6 agar slants were inoculated from a broth culture, and, after overnight incubation, were sealed with paraffin. A new slant was taken each month as the source of the organism. A broth subculture was made, and from that, a plain agar plate was streaked and incubated overnight. This served as the source of organisms for the cultures used that week. The present series of experiments was completed within a six-month period.

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RESULTS

Infectivity of different numbers of bacteria injected intravenously

The intravenous inoculation of 10^8 staphylococci in normal rabbits was followed by the death of over 50 per cent of the animals within 72 hours. All of those which survived 48 hours were found to have visible kidney infections, but no gross sign of infection was found in other organs.

The ease with which the rabbit kidney can be infected by staphylococci suggests that the kidney might be peculiarly susceptible to this organism. It was decided, therefore, to determine the size of inoculum necessary for renal infection by way of the blood stream. Serial 10-fold dilutions of culture were injected into a group of rabbits. The results are given in Table 1.

TABLE 1. RELATION OF SIZE OF INTRAVENOUS INOCULUM TO INCIDENCE OF RENAL INFECTION* IN NORMAL RABBITS

No. of organisms injected	*Incidence of renal infection†*
10^4	0/7
10^5	0/6
10^6	2/5
10^7	8/8
10^8	12/12

* The recovery of 10^5 or more staphylococci per kidney is taken to indicate infection. Those kidneys not infected contained fewer than 100 organisms. Gross abscesses were visible in most infected kidneys.

† Number of infections/number of tests made.

It can be seen that 10^6 organisms were the smallest inoculum which produced renal infection. This is comparable to the number of staphylococci needed to infect the mouse kidney[7] and is similar to the number of *E. coli* given intravenously necessary to infect the rat or rabbit kidney following ligation of the ureter.[5,9] The same relationship seems to pertain to enterococcal infections of the rat kidney.[8] It would appear then that a large number of organisms must be injected intravenously in order to produce infection of the kidney.

Staphylococci could be demonstrated in the blood of about three-fourths of the animals 24 hours after they had received 10^8 organisms. Urine cultures were positive only when there was kidney infection.

Kidney lesions produced by intravenous inoculation of staphylococci

The gross lesions found in infected kidneys were of three types: (1) Isolated disc-shaped abscesses, 1.0-2.0 mm. in diameter, similar in appear-

ance to a bacterial colony growing in a solid medium (Fig. 1). These lesions were found only in the medulla and were surrounded by a comparatively thick wall of inflammatory reaction. Although they appeared to obstruct nephrons draining through that area, there was little or no evidence of spread of infection throughout the obstructed nephrons in a manner similar to that observed in other forms of intrarenal hydronephrosis.[4, 12] (2) Wedges of infection with the apices in the papilla, forming confluent abscesses on the surface of the kidney (Fig. 2). (3) Severe papillary injury associated with wedge lesions, leading to necrosis of the papilla (Fig. 3).

Effect of age of culture on virulence

It has been demonstrated by Rogers that intravenous injection of an 18-hour culture of *E. coli* is lethal to a large percentage of rabbits, whereas the same number of organisms from a 4-hour culture produces neither death nor infection.[13] It was thought worthwhile, therefore, to determine whether the ability of staphylococci to infect rabbits varied according to age of the culture used. Using an inoculum of 10^8 organisms, no difference was found between a 4-hour and 18-hour culture in either the lethal effects or the renal lesions produced.

Effect of staphylococcal culture supernate on virulence of staphylococci and E. coli

The toxic effects of filtrates obtained from staphylococcus cultures are well known.[8] It seemed possible therefore that the ability of staphylococci, when given intravenously, to infect the normal kidney was dependent upon some effect of the simultaneously administered toxins.

To test this hypothesis, an 18-hour broth culture of staphylococci containing 10^8 bacteria per ml. was centrifuged at 3,500 revolutions per minute for 30 minutes at 10° C. This reduced the number of bacteria in the supernatant fluid to 10^6 organisms/ml. A 4-hour culture of *E. coli* (10^8/ml.) was similarly centrifuged, but the supernatant fluid in this instance was discarded. The *E. coli* were then resuspended in the original volume of staphylococcal supernate in a concentration to provide 10^8 *E. coli* per ml. Five-tenths ml. of this mixture was given intravenously to each of three rabbits. One week later the animals were sacrificed. All six kidneys appeared normal, but one of them yielded 10^5 staphylococci; the remainder were sterile. All urine cultures were negative save for the sample from the infected animal; this urine contained 10^5 staphylococci per ml.

This experiment tended to indicate that the simultaneous administration of staphylococcal culture medium did not enable *E. coli* to infect the

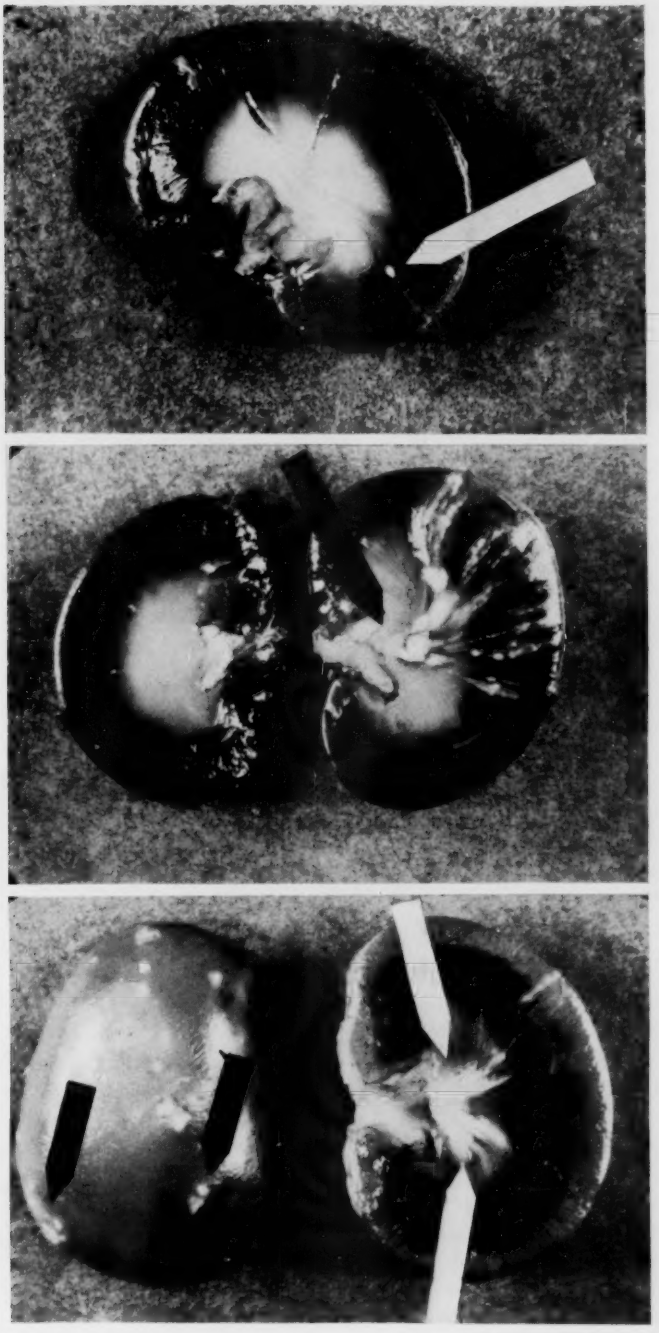

FIG. 1. Disc-shaped abscess resulting from the intravenous injection of 10^8 staphylococci.

FIG. 2. Wedge of infection resulting from the intravenous injection of 10^8 staphylococci. A normal kidney is demonstrated on the left.

FIG. 3. Papillary necrosis resulting from the intravenous injection of 10^8 staphylococci. Abscesses visible on the surface are shown on the left.

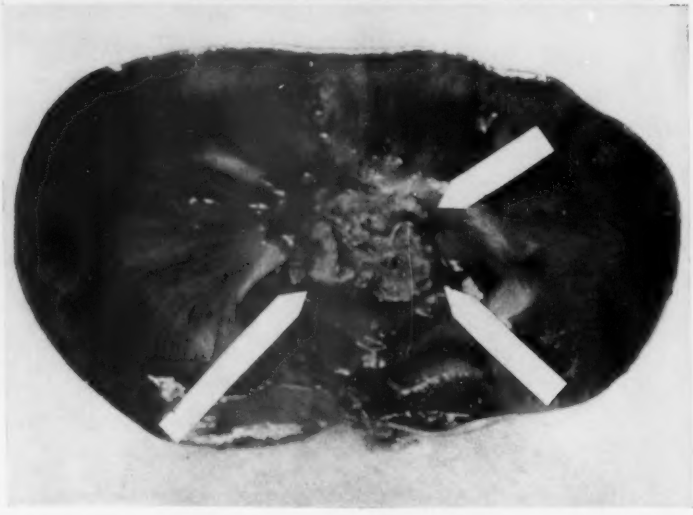

FIG. 4. Sterile linear scar one month after the direct injection of 10^4 staphylococci into the renal papilla.

FIG. 5. Renal stone one month after the direct injection of 10^4 staphylococci into the renal papilla.

normal kidney, nor was a relatively small inoculum of staphylococci able to produce more infections than it had in previous experiments.

In a related experiment, three rabbits were injected with *E. coli* centrifuged as before and resuspended in a 1:10 dilution of an 18-hour culture of staphylococci. This mixture contained the same number of *E. coli* with 1/10 the amount of staphylococcal culture medium and ten times as many staphylococci as in the previous experiment. One week after injection two animals had infection in one kidney, the remaining rabbit had bilateral renal infection. All urine cultures were positive. The positive cultures revealed staphylococci, but no *E. coli* could be identified.

TABLE 2. INCIDENCE OF INFECTION RESULTING FROM INJECTION OF BACTERIA INTO CORTEX OR MEDULLA OF THE NORMAL KIDNEY

No. of organisms injected	*Site of injection†*		*Colonies Staphylococci per kidney*
	Cortex	*Papilla*	
10		1/4	10^5
10^3		5/6	10^5, 10^5, 10^6, 10^6, 10^6
10^4	0/6		
10^5	1/3		10^5
10^6	1/1*		10^5
10^7	1/1*		10^5

* The contralateral kidney in these rabbits also became infected.
† Number of infections/number of tests made.

This experiment indicates that even when given at the same time as an infecting dose of staphylococci, *E. coli* infection cannot be detected. The findings are in agreement with those reported by Helmholtz.[10]

Direct inoculation of staphylococci into different zones of the kidney

Previously reported experiments with coliform bacteria had provided evidence of a great difference between the cortical and medullary zones of the kidney, in respect to susceptibility to infection. On direct inoculation, the number of bacteria required to infect the cortex was 100,000 or more; whereas as few as 10 organisms injected into the medulla were capable of causing infection. The same techniques were used to determine the number of staphylococci necessary to infect the two zones of the kidney. Animals were examined four days after injection of bacteria. The findings were similar to those which had been obtained with *E. coli*. See Table 2.

At least 10^5 staphylococci are required to establish infection when placed directly in the cortex of the kidney. The abscesses produced by cortical

inoculation of 10^5 staphylococci are localized to the area of injection and do not form wedges extending into the papilla. With larger numbers of organisms wedge-shaped infections are produced. These abscesses are, however, not limited to the site of injection but instead are found in both kidneys. It is evident that sufficient bacteria escaped into the blood stream at the time of injection to infect *both* kidneys.

In the case of the medulla, infection could easily be produced with small numbers of bacteria. An inoculum estimated to contain fewer than 10 viable units caused infection in one of four animals tested, and an inoculum containing between 10 and 100 viable units caused infection in five of six animals. The infection produced by the direct injection of staphylococci into the renal medulla also follows a course like that observed with *E. coli* infection. The process remains confined to a wedge of tissue in which tubular drainage had been interrupted by the needle puncture. This is remarkable in view of the capacity of staphylococci to infect the normal kidney via the blood stream. One might have expected the same infectivity to be apparent by either direct extension of infection through the kidney substance, or by the bathing of normal papillary tissue in heavily infected urine.

Rate of healing of staphylococcal abscesses

Further similarity between the lesions caused by the two organisms was found in the rate of healing of staphylococcal abscesses. Ten rabbits which had received papillary injections of from 10^2 to 10^4 staphylococci were examined two months afterward. Seven of them had but a single linear scar to mark the site of previous infection (Fig. 4), and cultures of whole kidneys and urine in these animals were sterile. In the remaining three, there was still an active infection, but this appeared to be related to the formation of renal calculi. These concretions, in the pelves of the kidneys, were yellow-brown, coarsely corrugated, oval in shape, with the longest dimension measuring 0.2-1.0 cm. (Fig. 5).

Inoculation of staphylococci into bladder urine

The staphylococcus used in the present experiments grows about as well in rabbit urine as it does in broth. It was decided therefore to test the effects of inoculating staphylococci into the bladder urine of 10 normal rabbits. Twelve to 14 days after the injection of 10^8 staphylococci the kidneys and bladders of all animals appeared normal. The left kidney of one rabbit contained 10^5 staphylococci, the remaining kidneys were sterile. In addition to the animal with the positive kidney culture, three other rab-

bits had staphylococci in the bladder urine. It is difficult to interpret the one positive kidney culture in an organ which appeared normal. Conceivably the bacteria were found in this organ as a result of reflux of infected urine from the bladder. It can be said at any rate that staphylococci inoculated into bladder urine are rarely able to establish infection in the normal kidney of the rabbit.

In other experiments, not published, we have found that coliform bacteria also do not infect the rabbit kidney following inoculation into bladder urine.

DISCUSSION

The present studies, utilizing a virulent strain of *Staphylococcus aureus,* have demonstrated that the susceptibility to infection of different zones of the kidney on direct inoculation of this organism is similar to that previously demonstrated for *E. coli;* infection is produced with as few as 10 organisms in the medulla, whereas approximately 100,000 are required to infect the cortex. The infections produced by the direct inoculation of either of these organisms are similar in appearance, method of spread and rate of healing. These results are surprising in view of the fact that when sufficient bacteria are injected into the blood stream of normal animals staphylococci regularly produce renal infection whereas the coliform bacteria do not. Factors such as age of the culture and the effect of culture fluid did not appear to be of importance. The question had to be considered whether transport through the blood stream either enhanced the virulence of the staphylococcus or diminished that of *E. coli.*

There is no information indicating that the virulence of staphylococci is enhanced when these organisms are inoculated into the blood stream. There is considerable evidence, however, which indicates that "avirulent" *E. coli* are more vulnerable to phagocytosis and destruction by leukocytes than are "virulent" staphylococci. This has been demonstrated *in vitro* by Cohn and Morse using normal rabbit leukocytes.[1] Of particular interest, however, are observations in normal rabbits[14] which demonstrate that following initial rapid clearance, staphylococci persist in the blood stream for many hours in a concentration 10 to 1,000 times that of *E. coli.* Some of our experiments in rats also lend support to this observation. Two hours following the injection of similar inocula, the number of staphylococci recoverable from the kidneys is 10 to 100 times as great as *E. coli.*[8] It is inconceivable that multiplication of staphylococci within two hours could account for this difference. Clearly then, staphylococci are much more

likely than coliform bacteria to arrive at the proper place in the kidney in a concentration sufficient to produce infection.

While one would expect to find great differences in pathogenicity of a "virulent" staphylococcus and an "avirulent" strain of coliform bacteria, the surprising result of these studies has been the remarkable similarities in their ability to produce infections when injected directly into the kidney. *This would seem to indicate that the factor most decisive in determining virulence for these two organisms is the effectiveness of host defense mechanisms as they operate in the circulating blood.*

Evidence has previously been presented pointing to the medulla of the kidney as the site of initial bacterial multiplication in experimentel pyelonephritis.[4, 12] The present work offers further confirmation of this thesis in the study where injection of 10^6 staphylococci into the cortex of one kidney produced wedge-shaped infection involving the medulla of both kidneys. A concentration of 10^5 bacteria per 0.05 ml. injected directly into the cortex had been shown necessary to produce local infection. This concentration of bacteria would not be achieved in the blood stream when only 10^6 organisms were injected. Since only 10 or fewer staphylococci per .05 ml. are required to produce medullary infection, it is likely that under these circumstances renal infection resulted from blood stream dissemination of organisms and had its beginning in the medulla of the same and the opposite kidney.

SUMMARY

Renal infection by the intravenous route in normal rabbits requires 10^6 or more virulent staphylococci. Direct injection of the organisms into the cortex and medulla of the kidney indicates a great difference in susceptibility of these two zones, since fewer than 10 bacteria produced infection in the medulla whereas 100,000 or more were necessary to cause an abscess in the cortex.

A virulent staphylococcus and the coliform bacteria differ in their capacity to infect the normal kidney following intravenous injection. Aside from this one feature, however, the present studies based on direct inoculation of bacteria into the kidney emphasize the remarkable similarities in the capacity of these organisms to produce infection. It is suggested that the difference in virulence demonstrable only upon intravenous inoculation is dependent upon the effectiveness of host defense mechanisms in the blood stream, thereby determining the fate of bacteria which have been injected and the proportion of the inoculum which is able to lodge in the kidney.

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A CONSIDERATION OF THE PATHOGENESIS OF BACTERIAL MENINGITIS: REVIEW OF EXPERIMENTAL AND CLINICAL STUDIES

INTRODUCTION

Fifty years ago bacterial meningitis was studied intensively in both laboratory and clinic. Many illustrious investigators—Councilman, Flexner, Austrian, Wollstein, and Weed, to mention a few—lent themselves to the task of elucidating the pathogenesis, anatomical changes, and pathological physiology associated with these infections and devising effective forms of treatment for this almost invariably fatal disease. With the advent of antibiotics the outlook for patients with meningeal infections improved considerably, and concomitantly studies on experimental meningitis almost disappeared from the literature. It is significant that in a recent symposium on the cerebrospinal fluid a discussion of experimental meningitis was conspicuously absent.[104] In contrast to most other bacterial infections, however, nearly one quarter of patients with meningitis die despite optimal therapy with antibiotics.[89] The reason for this persistent mortality is not clear but stems in part, at least, from inadequate knowledge of the manner in which microbes produce infection within the subarachnoid space—their portal of entry, rate of growth, mode of dissemination within neural structures, action upon the normal exchange of metabolites between blood and cerebrospinal fluid, effect upon essential enzyme systems, and the mechanism by which they produce death. In our present state of knowledge, answers to most of these questions are not available.

It is the purpose of this brief review to assemble the pertinent information on the pathogenesis and physiology of meningitis, correlate it with certain phenomena observed clinically and raise questions which may lead to sound basic and clinical investigation of the mysteries beclouding the nervous system's response to infection. We have purposely limited the dis-

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cussion to acute bacterial meningitis and have restricted the references to pertinent experimental papers, omitting most clinical reports. This does not imply, of course, that bacterial meningitis serves as the prototype for infections of the pia-arachnoid by other agents. It is hoped, however, that an analysis of the factors mediating meningeal infections by bacteria may stimulate further thought and experimentation on the effect of other injurious stimuli, biological or physical, on the coverings of the nervous system.

EXPERIMENTAL MENINGITIS

A. *General considerations.* The earliest attempts to produce experimental meningitis in animals utilized infected cerebrospinal fluid from patients to establish an etiologic relationship and to fulfil Koch's postulates.[15, 81, 41, 52, 110, 153, 186] After it had been demonstrated conclusively that a number of common pathogenic bacteria such as the pneumococcus, meningococcus, and *Hemophilus influenzae* were capable of causing lethal inflammation of the meninges, meningitis in animals was employed experimentally to evaluate therapeutic regimens including serum,[90, 92, 186] subarachnoid lavage with antiseptic agents,[130] antibiotics,[145, 173] and proteolytic enzymes.[84]

One of the difficulties in interpreting the mass of experimental data in this field, has been the failure to achieve a standardized, reproducible experimental model such as acute pyelonephritis in rats,[23] pneumococcal peritonitis in rabbits,[13] or staphylococcal disease in mice.[190]

Before embarking upon a review of the literature, it seems worthwhile to propose certain definite criteria for the ideal infection to use as a standard of reference for comparison with previous studies:

1. The portal of entry and route of dissemination of the organism must be akin to those in man.
2. The parasite must be pathogenic for man as well as experimental animals.
3. The course of the disease must be relatively predictable and of sufficient duration to permit evaluation of therapy.
4. The disease must be reproducible within the limits of biological variation.
5. The lesions in the experimental infection must be morphologically similar to those in man.
6. The technique must be relatively simple.

Unfortunately, no single investigation has fulfilled these requirements, and, indeed, many questions need be answered before the criteria enumerated can be met. Our lack of understanding concerning the pathogenesis

of infections of the nervous system has led to many artificial studies in which the investigator has designed the experimental setting to conform to the expected results. Many of these investigations, however, furnish valuable clues which may not always supply direct answers but provide topics for further discussion and experimentation.

B. *Relationship between route of infection and development of meningitis.* The hypothesis that bacteremia per se may be attended by meningitis was examined with a variety of organisms. The early claims of Bull[25, 28] and Elser and Hutoon[58] that meningitis follows the intravenous inoculation of bacteria have not been substantiated.[4, 7, 85, 181, 193] As might be expected, the more remote intraperitoneal route has also failed to yield meningeal infection.[1, 81, 116] A single attempt to infect the central nervous system by direct injection of organisms into the carotid artery was unsuccessful.[70]

A more rational approach, predicated upon the clinical finding that meningococci were frequently harbored in the nasal passages of patients with meningococcal meningitis, consisted of the direct application of bacteria to the nasal mucosa of animals. However, meningitis did not occur.[7, 15, 116] It has long been evident that infections of the paranasal sinuses, middle ear, and mastoids can spread to the meninges. Attempts to duplicate this phenomenon in cats were only intermittently successful.[200] Furthermore, even in this study physical irritants such as iodine and mustard oil were used to facilitate passage from the frontal sinus to the meninges. Osteomyelitis was a frequent accompaniment of sinusitis in this experimental model, and it is conceivable that the bone rather than the sinus cavity served as the infecting focus. Southard and Keene were able to infect the meninges by intraorbital inoculation of bacteria,[162] but it is not clear from their report whether the injections were intraorbital or, in fact, intracerebral.

These studies indicate that the nervous system is admirably protected from infection by microorganisms in the blood stream and do little to establish the importance of "neighborhood infection" in the pathogenesis of meningitis. Furthermore, neither method has provided a suitable model for evaluating therapy.

More success has followed injection of bacteria directly into the lumbar subarachnoid space. Of historic interest are the studies of Councilman *et al.* who infected a goat by this route.[41] Subsequently, other investigators have utilized this method with a variety of organisms and species.[7, 61, 85, 193] Even man has served as an accidental subject in experiments of this type when clinical meningitis has followed lumbar puncture for spinal anesthesia and intrathecal therapy.[48] The offending organism is

usually one of the Gram-negative enteric bacilli, chiefly *Pseudomonas aeruginosa*. Stanley has reported 32 cases of iatrogenic Pseudomonas meningitis with a mortality of 40 per cent.[188]

In experimental animals a number of difficulties have been inherent in the installation of organisms directly into the lumbar sac. Firstly, the lack of predictability of the course of infection made response to therapy difficult to ascertain.[92, 188] For example, Flexner, employing meningococcus, was impressed with the acute course of the disease.[61] On the other hand, Lamar[109] and Idzumi[85] were able to produce a protracted illness with the pneumococcus. Death in the acute cases was probably due to bacteremia, but bacteriological confirmation of this was sparse. Secondly, spinal cord injury has been frequent and bacteria have often been injected into the cord.[7, 85] Lastly, the small size of the lumbar sac in most species has led to inaccurate placement of bacterial inocula as well as a paucity of spinal fluid for serial studies.

Some of these objections can be overcome by employing suboccipital puncture with introduction of bacteria into the cisterna magna. The technique of cisternal puncture was first employed in animals by Dixon and Halliburton in 1913[48] and in man by Wegeforth *et al.*[193] several years later. Subsequently, this method was used extensively because of the accessibility of the subarachnoid space in this area and the ease with which repeated samples of spinal fluid can be obtained.[18, 19, 20, 56, 77, 97, 98, 104, 145, 166, 167, 173, 208] The main disadvantage of this experimental model is the frequent occurrence of bacteremia and early death,[56, 104, 145, 166, 173] raising the question whether death in these animals was a consequence of the inflammatory changes in the neuraxis or overwhelming sepsis. A similar problem may, in fact, exist in man, and Daniels has postulated that death in meningococcal meningitis was related to the "toxic" effect of bacteremia as much as meningitis.[45] This conflicts with the findings of Alexander *et al.*, who state that mortality in pneumococcal meningitis could not be correlated with bacteremic spread.[2] The prognostic implication of bacteremia in clinical meningitis deserves further study.

In animals, in addition to the acute septicemic form, the intracisternal instillation of bacteria may be followed by a chronic indolent infection of the meninges.[50, 167] The persistence of the inflammatory process may be related to subarachnoid blockage by large quantities of exudate or to the development of abscesses within the parenchyma of the spinal cord.[166] Since many of these infections were created for the purpose of analyzing some specific therapeutic regimen, the chronic exudative type is to be preferred to the acute septic type. Unfortunately, there is no way to predict

in the individual animal which course the infection will follow. By injecting ground jellied agar in combination with *Pseudomonas aeruginosa*, Hamburger was able to achieve a chronic exudative infection regularly.[77] Another group of animals infected intracisternally have had a relatively prolonged illness terminating in recovery or death. However, exudation was not a feature of their disease and at autopsy only minimal morphological changes were present in the nervous system but, interestingly enough, heart blood cultures were positive. Although endocarditis was not described, one wonders whether it may have been present. The course of these animals is reminiscent of that of patients who recover from pneumococcal meningitis only to succumb to endocarditis.[8]

Minor difficulties which have been encountered with the intracisternal route are extravasation of the inoculum into the surrounding soft tissues and direct trauma to the medulla oblongata.[142]

Intradural administration of bacteria through a trephine is another technique for infecting the meninges.[15, 100] This method affords increased accuracy by permitting direct visualization of the meninges but is somewhat tedious. Manwaring attempted placement of virulent tubercle bacilli into the basilar cisterns by puncturing the frontal lobes with a long needle.[135] As pointed out by Austrian,[8] however, this maneuver was accompanied by extensive damage to cerebral parenchyma.

Compared to its extensive use in the investigation of neurotropic viruses, direct intracerebral inoculation of bacteria has been used rarely.[64, 70, 124, 130] In most animals, either transient neurological disturbances or a diffuse cerebritis with fulminating septicemia are observed. Localization of the infection with abscess formation was possible only when the cerebral parenchyma was injured by needle puncture several days before bacteria were reinjected into the site of previous trauma.[70, 124, 130]

Flexner and Amoss performed a series of classical experiments in monkeys in which the combination of sterile aseptic meningitis and viremia was used to induce clinical poliomyelitis.[60, 65] In a number of studies in which bacteria rather than viruses were given intravenously following aseptic meningitis, meningeal infection occurred only sporadically.[4, 7, 85, 121] Sterile inflammation of the meninges can regularly be observed following the intrathecal administration of saline, serum, and a variety of other substances and probably enhances the passage of pathogens from blood to nervous system. Clinically it appears to be of little significance since bacterial superinfection in patients with aseptic meningitis is rare.

The studies described thus far do not elucidate the train of events in the pathogenesis of meningeal infections in man and, except for meningitis

secondary to a neighboring focus and iatrogenic infection following lumbar puncture, have little applicability to human disease. While there is no direct evidence that microorganisms reach the meninges via the blood stream, the frequency with which meningeal infection is preceded by bacteremia suggests that the hematogenous route may be an important pathway by which bacteria reach the nervous system. This thesis gains support in the studies of Weed *et al.*, relating alterations in cerebrospinal fluid dynamics during experimental bacteremia to the development of meningitis.[179, 181] When cerebrospinal fluid was removed from cats immediately preceding or following the intravenous introduction of a virulent culture of *B. lactis aerogenes,* generalized leptomeningitis developed, usually within 24 hours. Control animals given twice the intravenous dose invariably remained well. *B. lactis aerogenes* was used in the majority of experiments, but infection could be produced by several other bacterial species. This phenomenon did not appear to be peculiar to the cat since other animals could also be infected.

The obvious explanation for these results is that trauma to small meningeal blood vessels at the time of lumbar or cisternal puncture provided access for bacteria but careful morphological studies failed to substantiate this.[9] Furthermore, infusion of hypertonic solutions, compression of the jugular veins, and transient cardiac standstill during bacteremia were also followed by meningitis, indicating that direct injury to nervous structures was not a sine qua non in the development of this infection.[181] Weed postulated that the occurrence of infection was facilitated by the transient lowering of cerebrospinal pressure associated with these procedures. Unfortunately, there are no data to support this hypothesis because no accurate measurements of spinal fluid pressure were made. Since jugular compression, which is usually attended by an abrupt rise in cerebrospinal fluid pressure, had the same effect as procedures which presumably resulted in cerebrospinal hypotension, one wonders whether the rate of change rather than the direction may not be of importance. A crucial experiment would be to determine whether the re-injection of cerebrospinal fluid following its removal during bacteremia would prevent meningeal infection. Unfortunately, data on this point are scanty.

The applicability of Weed's experiments to the pathogenesis of the disease in man is difficult if not impossible to determine. Even if it were postulated that bacteremia antedated meningitis under natural conditions, the magnitude of change in spinal fluid dynamics might be insufficient to facilitate infection. Of more clinical significance is the possible danger of diagnostic lumbar puncture during bacteremia. Wegeforth and Latham per-

formed lumbar punctures in 93 patients with suspected meningitis.[184] In 55 of these, the cerebrospinal fluid was normal. Six patients had meningococcemia or pneumococcal bacteremia at the time of lumbar puncture, and five of the six subsequently developed meningitis. On the basis of these findings, the authors condemn diagnostic lumbar puncture in the absence of strong clinical indications. Remsen shared this viewpoint and added a case of streptococcal meningitis occurring under similar circumstances.[153] It seems likely that these few cases are not representative of the over-all clinical experience, and that the occurrence of meningitis following lumbar puncture during bacteremia is relatively rare. Pray lends support to this by presenting evidence that the incidence of meningitis in children with pneumococcal bacteremia is not increased by lumbar puncture.[140] Compared to the risk of missing the diagnosis of meningitis by omission of lumbar puncture, the chance of developing meningitis following the procedure is small even in the presence of bacteremia, and there are not sufficient data to consider "suspected or documented sepsis" as a contraindication to lumbar puncture.

Although not strictly pertinent to the subject of acute bacterial meningitis, the studies of Rich and McCordock based upon some of these early negative experiments deserve mention.[181] These investigators noted a rather persistent discrepancy between the age of the visceral lesions in miliary tuberculosis and the tubercles in the meninges, the latter appearing more acute than the former. This finding suggested to them that the meninges were probably not seeded with tubercle bacilli by the hematogenous route. Furthermore, they were consistently unable to produce meningitis by the intravenous injection of virulent tubercle bacilli into hypersensitive animals despite the fact that massive visceral tuberculosis invariably developed. On the other hand, intrathecal administration of acid-fast organisms resulted in infection of the meninges. These experiments indicated that the pia-arachnoid was infected from contiguous foci in the underlying cerebral parenchyma, and this impression was confirmed by the finding of tuberculomata in the cerebral cortices of 77 of 82 patients with tuberculous meningitis. Unfortunately, no such clinicopathological relationships have been derived in acute bacterial meningitis, and it is not known whether infected CSF serves as a focus for infection of the brain and spinal cord or whether the reverse situation obtains.

C. *Causative pathogen.* Meningitis in animals has been produced experimentally by most of the organisms which cause the disease in man. The common human pathogens *D. pneumoniae, N. intracellularis, H. influenzae,*

and *Staph. aureus* have been used most often. Gram-negative enteric bacilli have been the subject of a few studies.[36, 77, 181]

In man there is good correlation between the etiological pathogen and the clinical setting. Thus, pneumococcal meningitis is frequently a sequel to infection of the paranasal sinuses, mastoids, and pleural space; the meningococcus tends to produce a fulminating illness in an epidemic setting; hemophilus shows predilection for children, staphylococcal meningitis is prone to occur with cavernous sinus thrombosis or epidural abscess, and Klebsiella meningitis is most often seen in diabetics or after head trauma.[162a, 173a] Similar differences have been noted in some animal experiments. Although different animals were employed, pneumococcal meningitis[85, 100] appears to run a more protracted course than the disease produced by the meningococcus.[61] Stewart found that type II pneumococcal meningitis was characterized by formation of a heavy fibrin exudate and rapid development of subarachnoid block, while type I caused a septicemic illness associated with minimal exudation.[107, 160]

Most cases of iatrogenic meningitis are caused by Gram-negative organisms rather than other human commensals.[48] This observation in conjunction with experimental evidence suggests that Gram-negative bacteria may possess a high degree of virulence for the meninges.[58] The capacity of these organisms to elaborate an endotoxin may account for the increase in virulence. Pertinent to this point are the studies of Branham and co-workers in rabbits which suggest that the damage to the meninges following injection by the intracisternal route may be due to a toxic product of the pathogenic bacterium rather than the intact organism.[18–21] They found that purulent or fibrinopurulent meningitis occurred with living meningococci as well as organisms which had been boiled or passed through a Berkefeld N filter and they were unable to distinguish clinically or pathologically between the infections produced by the different preparations. Direct instillation of highly purified endotoxin from *Salmonella abortus equi* and *Shigella flexneri (type Z)* into the cisterna magna of dogs also resulted in marked cerebrospinal fluid pleocytosis.[142]

The virulence of the infecting organism is enhanced by serial intrameningeal passage.[4, 97, 145, 173] In this respect the meninges resemble other tissues. This phenomenon is independent of the species and organism employed. Its clinical significance in man is unknown.

D. *Susceptible host.* Meningitis occurs naturally in a wide variety of animals. For example, spontaneous meningococcal and pneumococcal meningitis has been described in horses, calves, and goats; young animals appear to be more vulnerable.[84] Pseudomonas has been incriminated as a cause

of meningitis in pigs with infectious rhinitis.[108] In view of the wide natural host range of meningeal infections, it is not surprising that the disease has been produced with ease in guinea pigs, rabbits, monkeys, dogs, and cats. The last two lend themselves well to experimentation. Their relatively large size minimizes the difficulty of lumbar puncture encountered in smaller species and also furnishes an adequate amount of spinal fluid for examination. Rabbits are poor experimental subjects since many have sustained cord damage at time of lumbar puncture and cerebrospinal fluid has been difficult to obtain in sufficient quantity. This species is also highly susceptible to the lethal effect of bacteremia with certain organisms.[7, 85, 106]

Different species may be particularly resistant to bacterial infection with certain organisms. For example, the dog tolerates pneumococcal meningitis better than the rabbit.[85] There is little other information concerning differences in the susceptibility of individual species to infection of the meninges.

THE EFFECT OF INFLAMMATION ON THE PERMEABILITY OF THE MENINGES

A. *General considerations.* The mechanisms controlling the passage of substances in and out of the nervous system are complex and poorly understood. Functionally, two sites of exchange have been postulated. One, which encompasses the entire neuraxis, is concerned with a transfer of organic and inorganic materials between the plasma and the cerebrospinal fluid—the blood-cerebrospinal fluid barrier. The other, termed the blood-brain barrier, is the locus of interchange between the blood stream and neural parenchyma. The anatomical location of these two barriers, their relationship to one another, and their basic physiological actions are controversial and not germane to the present discussion. Suffice it to say, both are profoundly altered by inflammation of the meninges. These changes in permeability may contribute to the aberrations of the spinal fluid in meningitis and may affect the course of the disease. Finally, the passage of drugs into the nervous system is controlled to a large extent by these barriers and any variation in the entry of chemotherapeutic agents is of obvious importance. In the following section, the effect of inflammation on the permeability of these barriers to a variety of exogenous and endogenous substances will be discussed.

B. *Exogenous substances.* 1. Inorganic ions. A number of inorganic substances normally not found in the spinal fluid have been given to patients and animals with meningitis to determine whether they could be recovered in the spinal fluid. Of the many anions tested,[97] studies with bromide[86, 177] and radioactive phosphorus[58] have been most valuable. The bromide test

involves the oral or intravenous administration of sodium bromide followed by simultaneous measurements of this ion in the serum and spinal fluid after equilibrium is established. Normally the ratio between blood and cerebrospinal fluid bromide is 2.6 and remains remarkably constant. With the development of meningitis, the ratio approaches unity and rises again as healing takes place.[160, 172]

The distribution of radioactive phosphorus (P^{32}) has been measured in experimental staphylococcal and streptococcal meningitis in rabbits.[58] Within 24 to 36 hours after the induction of infection, the P^{32} concentration in the cerebrospinal fluid of animals with meningitis was three times that of the controls. The P^{32} concentration in the cerebral parenchyma had risen two-fold.

2. Dyes. The historic observation of Goldmann that trypan blue given parenterally was unable to penetrate into the nervous system initiated the concept of the blood-brain barrier and prompted investigation of a number of organic dyes as indicators of meningeal permeability.[72] Koyama reviewed the passage of 52 dyes into the cerebrospinal fluid of rabbits and found that their concentration was increased in meningitis.[107]

Sodium fluorescein (Uranin) also lends itself to measurement of the integrity of the blood-cerebrospinal fluid barrier. Following oral or parenteral administration, little is found under normal conditions, but it is readily detected in patients with bacterial or tuberculous meningitis.[17, 58, 86, 111] Similar results have been reported in syphilis of the nervous system.[137]

Of interest are a few experiments in which organic dyes were utilized to determine passage from the spinal fluid to the blood, and it is noteworthy that some substances which gain access from blood to cerebrospinal fluid with difficulty pass in the reverse direction with ease. Thus, trypan blue given intrathecally is detectable in the blood stream shortly after instillation.[73] Similarly, normal infants given phenolsulfonphthalein (PSP) intrathecally excrete the dye in the urine shortly thereafter.[44] Limited studies suggested that intrathecal PSP may be retained in the subarachnoid space when the meninges become involved by disease.[126] This might be of clinical significance since therapeutic agents might be handled in similar fashion.

3. Toxic products of bacteria. It is conceivable that the ability of bacteria to cause infections in the nervous system is related not to their invasiveness but rather to the ability to elaborate endotoxins which might facilitate their passage from blood to brain. This may account for the meningeal virulence of *B. lactis aerogenes* described by Weed, and, more importantly, for the propensity of *Neisseria intracellularis* to cause meningitis.

The intracarotid administration of endotoxin has been shown to enhance the passage of fluorescein into the brain of the rabbit.[81] When the toxin was given intravenously, there was also some increase in passage of the dye from blood to CSF although this occurred only after a lag period of two hours.[144] On the other hand, administration of typhoid vaccine to dogs four hours preceding the intravenous injection of pneumococci, followed by lumbar puncture, did not increase the incidence of pneumococcal meningitis in these animals.[149] These experiments are far from conclusive, and the role of endotoxins in the pathogenesis of bacterial meningitis deserves further study. In contrast to endotoxins of Gram-negative bacteria, the exotoxins of diphtheria, tetanus, and staphylococcus were not injurious to the blood-brain barrier.[29]

4. Drugs. The passage of drugs into the spinal fluid under normal and abnormal conditions has received intensive study. The present discussion will limit itself to the passage of antimicrobial agents in meningitis. Aseptic meningitis facilitated the passage of arsphenamine into the cerebrospinal fluid[139] but did not enhance its deposition in the brain or spinal cord.[105] Binding by plasma proteins limits the ingress of sulfonamides into the spinal fluid, but usually sufficient quantities enter to achieve theoretically adequate therapeutic levels.[78] Sulfanilamide penetrates into the cerebrospinal fluid most effectively, and, after equilibrium has taken place, the cerebrospinal fluid concentration is equal to that of the blood.[78]

Since penicillin is the drug of choice in the treatment of Gram-positive coccal meningitis, its passage into the cerebrospinal fluid has received wide attention. Many studies employing small dosages of the drug attest to the relative impermeability of the blood-cerebrospinal fluid barrier to penicillin.[89, 90, 121, 149] When large amounts of penicillin are given parenterally, there seems to be a slight increment in the amount of drug found in the cerebrospinal fluid, but this is not a linear relationship.[16, 149, 154] The data provided by these studies are limited and require confirmation. Fortunately, there is general agreement that transfer of penicillin from blood to spinal fluid is increased in meningitis.[89, 90, 154] Little is known about cerebrospinal fluid level of the drug during acute infections, but the striking decrease in mortality observed with the use of this drug provides prima facie evidence that in the majority of cases therapeutically effective concentrations are reached. Based on a comparison with several series of patients with pneumococcal meningitis treated with parenteral penicillin it can be concluded that the addition of the drug intrathecally does not exert a major effect.[27, 118, 150] However, in occasional patients with peripheral circulatory collapse, intrathecal administration may offer a distinct advantage.

Under normal conditions streptomycin does not diffuse readily into the cerebrospinal fluid, but its passage is enhanced by meningeal inflammation.[24, 79, 201] Although it was once considered necessary to administer streptomycin intrathecally, this practice is distinctly hazardous and should be avoided.[20, 191] Fortunately, the advent of isoniazid in the treatment of tuberculous meningitis and chloramphenicol for infections caused by *H. influenzae* has led most clinicians to abandon the use of intrathecal streptomycin. There is little doubt, however, that prior to utilization of massive parenteral chemotherapy, and the advent of more effective drugs, the direct instillation of antimicrobials into the subarachnoid space was often lifesaving.[198a]

The tetracyclines are capable of traversing the blood-cerebrospinal fluid barrier, tetracycline being the most diffusable and oxytetracycline the least.[198] Normally, the tetracycline level in the cerebrospinal fluid is one-tenth of that in the blood.[190] Infection enhances the permeability of the meninges to oxytetracycline and, presumably, to the others in this group.[198]

Chloramphenicol is regularly detected in the cerebrospinal fluid when blood levels greater than ten micrograms per ml. are reached, and the concentration in the spinal fluid is approximately 25 per cent of that in the blood.[155] The availability of chloramphenicol as the highly soluble succinate salt makes blood levels of this magnitude easily attainable.

Bacitracin diffuses readily when the meninges are inflamed but poorly when they are normal.[178] In contrast, isoniazid is widely distributed throughout the body including the spinal fluid.[188] Measurement in a single patient with tuberculous meningitis suggested that hydrocortisone may enter the cerebrospinal fluid more readily than normal in this infection.[42]

The levels of various drugs in the CSF should not be equated with those of nervous tissue. A few studies which measure the passage of drugs into the cerebral parenchyma have been done[108, 189] but simultaneous measurements of drug levels in brain and cerebrospinal fluid have been limited and more are needed.

C. *Endogenous substances.* As has been pointed out, inflammation not only promotes the transport of foreign materials into the nervous system, but also disturbs the equilibrium between substances normally present in blood and spinal fluid. Among these are protein, sugars, organic acids, and various inorganic ions.

1. Protein. Elevation of the cerebrospinal fluid protein is typical of bacterial meningitis. The nature and origin of this protein have not been clearly defined. In the normal dog, there is active exchange of radioactive iodinated serum albumin between the plasma protein pool and the spinal

fluid.[59] These studies have not been performed in patients or animals with bacterial meningitis. However, electrophoretic studies demonstrating an increase in the cerebrospinal fluid albumin fraction in the early stages of human meningitis suggest that infection increases meningeal permeability to serum protein.[101, 112, 127] As the infection progressed, increases were noted in all fractions of the cerebrospinal fluid globulin; with subsidence of meningitis, the elevation became localized to the gamma globulin fraction.[127]

Increased vascular permeability and cellular breakdown have been held to be the cause of the protein elevation in meningitis.[69] On the other hand, a portion of this protein may emanate from nervous tissue per se. In support of this idea are the experiments of Mutermilch demonstrating the capacity of the nervous system to form antibodies to sheep red cells given intrathecally.[125, 126] Furthermore, elevation of the CSF gamma globulin without concommitant increase of serum gamma globulin has been reported in multiple sclerosis and central nervous system lues.[92, 100] The increased cerebrospinal fluid gamma globulin found late in meningitis may represent an immune response of the nervous system.

Pertinent to this point is the observation that seronegative patients with tuberculous, meningococcal, and lymphocytic nonbacterial meningitis occasionally have a transiently positive Wasserman test in the CSF.[155a] This suggests that under certain conditions neural tissues may elaborate a reagin which combines with alcoholic beef heart extract. This hypothesis would be more convincing were it not for the fact that some patients with seropositive latent lues also develop positive CSF complement fixation tests for syphilis during the course of bacterial or viral meningitis. In these circumstances, passage of the reagin from blood to CSF must surely be a consequence of a break in the blood-CSF barrier.

2. Sugar. A depressed spinal fluid sugar is the hall-mark of bacterial meningitis. Hypoglycorrhachia of lesser degree has been described in tuberculosis, syphilitic[123] and yeast meningitis, sarcoidosis of the nervous system,[140] diffuse leptomeningeal carcinomatosis,[14, 60, 170] and subarachnoid hemorrhage.[123] The low cerebrospinal fluid sugar is the single most important difference between tuberculous and viral meningitis.

Normally the level of glucose in the spinal fluid closely parallels that in the blood.[46, 67] For example, artificially induced hyperglycemia in man and animals is accompanied by a rise in the cerebrospinal fluid sugar. It is noteworthy, however, that the maximum increase in the cerebrospinal fluid glucose is not observed for several hours following hyperglycemia.[30, 75, 126] Conversely, hypoglycemia is associated with a fall in cerebrospinal fluid glucose.[46, 94]

The mechanism governing the disappearance of cerebrospinal fluid sugar in bacterial meningitis has not been clearly elucidated, and the relative importance of cells, bacteria, changes in meningeal permeability, and accelerated utilization by neural tissue in the genesis of hypoglycorrhachia remains poorly defined. On the basis of existing evidence, however, certain categorical statements can be made.

1. Clinical and experimental bacterial meningitis is characterized by a pleocytosis, presence of bacteria, and a depression of spinal fluid sugar.
2. This fall in cerebrospinal fluid sugar is not observed in aseptic or viral meningitis despite the presence of leukocytes. This has been established both in animals,[10, 74, 80] and in man.[175]
3. Incubation of sterile, cell-free spinal fluid at room and body temperatures for prolonged periods has not been attended by a decrease in sugar content.[10, 27, 55, 81, 82, 98, 122, 134, 138]
4. The addition of cells to sterile cerebrospinal fluid *in vitro* reduces the amount of available sugar,[10, 74, 81, 82, 86, 122, 161] and the degree of glycolysis is directly proportional to the number of cells. Polymorphonuclear leukocytes appear to utilize glucose more avidly than lympocytes.[81, 122, 161]
5. When pneumococci were incubated in cell-free, sterile spinal fluid, no glycolysis occurred unless the number of organisms far exceeded that present in experimental infection in dogs.[74] The *in vitro* utilization of glucose in cerebrospinal fluid by several other bacterial species has yielded inconsistent results.[10, 81, 98, 122]

The discrepancy between the normal sugar levels in aseptic meningitis and the capacity of leukocytes to utilize sugar *in vitro* has led to the suggestion that the inflammatory process makes the meninges more permeable to glucose. This in turn permits replenishment of cerebrospinal fluid glucose from the blood. Another explanation which comes to mind is that the exudate in most instances of aseptic meningitis consists predominantly of lymphocytes which use but little glucose.

In view of the fact that bacteria are essential for hypoglycorrhachia *in vivo*, it seems anomalous that the number of organisms necessary to produce a comparable fall *in vitro* does not manifest itself *in vivo*. On the basis of these experiments, one cannot attribute the decreased sugar in meningitis to either cells or bacteria alone. It is conceivable that the glycolytic power of bacteria may be enhanced by the presence of cells. This has not been tested under experimental conditions *in vitro*. That it may occur, however, can be discerned from recent studies of Petersdorf *et al.*

who produced pneumococcal meningitis in dogs made leukopenic by total body irradiation.[168] These animals were unable to respond to the meningeal infection with a pleocytosis and experienced no fall in cerebrospinal fluid sugar despite the presence of a large number of organisms. Changes in the permeability of the blood-cerebrospinal fluid barrier in this situation are probably of minor consequence.

Some evidence exists that the blood-cerebrospinal fluid barrier may be altered in the course of tuberculous meningitis. This is not unexpected in the face of the slow utilization of glucose by the tubercle bacillus[117] and the predominantly lymphocytic response. Furthermore, sugar introduced intrathecally into patients with tuberculous meningitis disappears more rapidly from the spinal fluid than could be explained by utilization by cells or acid-fast bacilli.[130, 178, 185] These investigations imply more rapid transfer of sugar from the spinal fluid to the blood stream. It has also been noted that hyperglycemia in tuberculous meningitis is paralleled by a rapid rise in cerebrospinal fluid glucose, connoting a disruption of the blood-cerebrospinal fluid barrier.[110]

3. Organic metabolites. Lactic acid in the spinal fluid rises in purulent meningitis and a variety of other conditions.[73, 130, 197, 198] The elevation appears to vary directly with the cell count and correlates poorly with reduction in the cerebrospinal fluid glucose. It seems likely that the organic acid is a by-product of cellular breakdown.[47, 105]

Ethyl alcohol is increased in the spinal fluid of some patients with cryptococcal meningitis who also have a low cerebrospinal fluid sugar.[174] This ingenious observation, which facilitates the differentiation between tuberculous and cryptococcal meningitis, is based on the fact that ethanol is the end product of glycolysis by yeast forms.

4. Inorganic ions. The decrease in the cerebrospinal fluid chloride in meningitis is probably a reflection of the loss of this anion from the extracellular fluid since the ratio of cerebrospinal fluid to blood chloride remains fixed.[115, 198] Cheek has suggested that the hypochloremia in tuberculous meningitis is a consequence of metabolic alkalosis and hypotonic expansion of the extracellular compartment.[22, 23]

The concentration of sodium in the spinal fluid is also dependent upon the serum concentration. Experimental hypernatremia results in a rise in cerebrospinal fluid sodium.[40] This may be a reflection of increased permeability since it has been demonstrated that hypernatremia in cats has been associated with profound damage to neuronal tissue.[218] Further support for enhanced passage of sodium into the spinal fluid following meningeal injury may be adduced from the finding that cerebrospinal fluid levels

of radioactive sodium in tuberculous meningitis are higher than in controls.[13] In contrast to sodium, potassium in the spinal fluid does not vary with alterations of the serum potassium.[40] This cation has not been studied in meningitis.

Elevations in the cerebrospinal fluid phosphorus have been reported in meningitis,[85, 86, 121] as well as in a variety of other neurological disorders.[70] Its significance is not known. There is a decrease in magnesium in the cerebrospinal fluid of patients with chronic meningitis,[119] but calcium is elevated.[86, 121] Both ionized and protein-bound calcium contribute to this rise.

THE ROLE OF HOST FACTORS IN MENINGITIS

A. *Anatomical and physiological defense mechanisms.* Compared to other bacterial infections, meningitis is a rare disease, and bacterial pathogens isolated in meningitic infections much more frequently parasitize other sites in the body. Of the bacteria most commonly found in meningitis only *Neisseria intracellularis* exhibits particular affinity for the nervous system. However, this organism is most often a benign commensal in the upper respiratory tract and only rarely is a cause of clinical infection. The implications of these clinical observations are that the central nervous system is extraordinarily well protected from infections, a most fortunate circumstance considering the high mortality and disastrous sequelae of meningitis. Not the least important of these protective mechanisms is the anatomical position of the nervous system. Encased by bone and connective tissue, it is well shielded from external stimuli, including bacteria. In many instances of meningitis, a specific disruption of this anatomical barrier such as fracture, osteomyelitis, or dural tear can be demonstrated.

Functionally, the nervous system is also protected from invasion of bacteria by unique filtering mechanisms—the blood-CSF and blood-brain barriers. As has been mentioned, these barriers are poorly defined anatomically and physiologically but there is little question about the remarkable control which they exert upon the passage of metabolites into and out of the nervous system. At the same time the entry of noxious substances appears to be prevented. The precise alterations in these barriers in response to infection remain ill defined but are probably most important.

B. *Susceptibility of neuronal tissue.* Little is known about the susceptibility of neuronal tissue to bacterial infection. It is not known, for example, whether there are factors indigenous to the cellular elements in the brain, spinal cord, and meninges which make them react in a particular manner to certain species of bacteria. *B. lactis aerogenes* has been said to be particu-

larly virulent for the meninges, but there is no evidence based upon *in vitro* studies that this tissue supports the growth of the pathogen better than others elsewhere in the body. Although poorly phagocytic normally, the arachnoid lining cells are capable of transformation into effective phagocytes under conditions of extreme irritation.[182] The nature of these irritating stimuli has not been elucidated and, in general, little is known about the role of phagocytosis in infections of the meninges. Lysozyme in the CSF is known to be increased in bacterial meningitis; this is probably merely a reflection of the presence of polymorphonuclear leukocytes, which are rich in this enzyme.[147] It is conceivable, however, that lysozyme is a product of injury to nerve cells per se.

C. *The inflammatory reaction in the meninges.* While it seems clear that there are potent defense mechanisms to prevent the entry of pathogenic bacteria into the CNS, once infection is established host defenses are quite inadequate. Proof of this statement lies in the high mortality prevalent prior to the use of antibiotics. Even with appropriate chemotherapy and sterilization of the CSF, the mortality rate remains close to 25 per cent. The mechanism of death in meningitis under these circumstances is not well understood. Endocarditis and pneumonia may account for some therapeutic failures; the majority, however, are probably related to the fact that the inflammatory reaction, normally an important cog in the defense to infection, may be distinctly deleterious in the cranial cavity. In most loci the inflammatory exudate or pus is easily removed by drainage, expectoration, or micturition depending upon the site of the infection. These avenues of escape are not available in acute leptomeningitis, and resolution and absorption of inflammatory cells are the only means for recovery. Failure to remove the exudate may result in an increase in intracranial pressure or development of subarachnoid block, either of which can be fatal. Inflammatory exudates may also adversely influence the action of antibiotics; Wood and Smith demonstrated in experimental pneumococcal pneumonia that antimicrobial activity was greater at the periphery of the lesion, where bacteria were actively multiplying and cellular exudation was sparse, than in the center loaded with masses of inflammatory cells and dead organisms.[195]

ACTH and cortisone have been employed in bacterial meningitis to mitigate the inflammatory response and to prevent some of these complications. Ribble and Braude reported recovery in 11 of 12 patients with pneumococcal meningitis treated with penicillin and adrenal cortical hormones, a therapeutic result superior to any group treated with penicillin alone.[150] However, this study contained no controls. More recently, Lepper and Spies treated alternate patients with pneumococcal meningitis with adrenal steroids and penicillin and were unable to demonstrate that the hormones exerted a

beneficial effect.[114] There seems to be little doubt that adrenal steroids have favorably affected the course of tuberculous meningitis and should be used in addition to chemotherapy.[90] In the face of these inconclusive data the effectiveness of adrenal hormones as therapeutic adjuncts must remain *sub judice*. There is good theoretical basis for their use not only to minimize the inflammatory response but also to modify the acute hypersensitivity reaction to bacterial products which may play a role in some types of bacterial meningitis (*vide infra*). Further therapeutic trials with adrenal hormones seem indicated particularly since it appears that they are not harmful in acute bacterial infections of man when proper antibiotics are administered.[176]

The instillation of the proteolytic enzymes, streptokinase-dornase, into the subarachnoid space to aid in resolution of the thick proteinaceous exudate has received wide attention. The results have not been impressive and sometimes the constitutional reaction to these drugs has been extraordinarily severe. In a recent enthusiastic report Tillet described the action of these enzymes in pneumococcal meningitis.[88]

D. *The allergic response of the meninges.* The capacity of the meninges to manifest "delayed hypersensitivity" in the form of an acute exudative reaction suggests that this may be an important mechanism in the development of increased intracranial pressure and subarachnoid block. Burn and Finley produced a severe exudative meningitis in guinea pigs with visceral tuberculosis by the intrathecal administration of tuberculoprotein; normal controls did not become ill.[28] These experiments were the forerunner of those of Swithbank, Smith, and Vollman who described an acute nonbacterial meningitis in Mantoux-positive patients given PPD intrathecally.[171] Clinically, these patients, who were described as having an "intrathecal tuberculin reaction," developed fever, headache, and nuchal rigidity. The CSF showed pleocytosis and elevation in protein, and the blood-CSF barrier became more permeable to bromide.[160] The intensity of the response was directly proportional to the amount of tuberculin administered and the sensitivity of the host; Mantoux-negative individuals did not develop meningitis, and, conversely, the severity of the reaction was ameliorated following desensitization. There is little doubt that the intrathecal tuberculin reaction is an allergic response of the meninges. Whether it of any importance in tuberculous meningitis or whether delayed hypersensitivity to other bacteria is responsible for the formation of exudate in the subarachnoid space is not known. Exudation produced in this manner may not be entirely detrimental since, in some instances at least, intrathecal tuberculin has been effective in relieving subarachnoid block in patients with tuberculous meningitis.[5, 159]

E. *Immunity*. The role of specific immunity in bacterial meningitis has been studied extensively. In the pre-antibiotic era, introduction of specific antisera into the subarachnoid space was of some benefit in experimental meningococcal, pneumococcal, and hemophilus meningitis.[60, 68, 109, 186] With the exception of some enthusiastic reports,[51a, 197] the results in man were unimpressive.[96a] There is some evidence that the combination of specific rabbit antiserum and sulfonamides exerted a synergistic effect in hemophilus infection of the meninges.[3a]

Normally, there is little or no transfer of antibody from the blood into the spinal fluid.[11, 80, 104] Using a more sensitive technique, Freund demonstrated antibody in the CSF of rabbits actively and passively immunized with typhoid bacilli.[60] The ratio of blood to CSF antibody titers in actively immunized animals was 300 : 1, which was quantitatively similar to the ratio of serum to CSF gamma globulin determined by electrophoretic techniques.[91] The blood-CSF and blood-brain barriers may differ in their permeability to immune particles, and the localization of antibody in the brain is not necessarily accompanied by a rise of the same antibody in the spinal fluid.[69, 71]

Passage of antibody into the CSF is enhanced during aseptic meningitis and has been demonstrated for neutralizing substances to poliomyelitis[94, 96] and meningococcal agglutinins.[8] Similarly, hemolysins to sheep red cells and complement have also been found in the CSF of patients with meningitis, but not in normals.[197, 198]

In view of the precipitous onset and brief duration of bacterial meningitides, active immunity may not be acquired in time to influence the outcome of these acute infections. Furthermore, as in subacute bacterial endocarditis, patients with bacterial meningitis may have antibodies to the offending pathogen in the circulation. This suggests that antibody does not reach adequate concentration in the CSF, even in meningitis. An alternative explanation, suggested by the relative ineffectiveness of antisera therapeutically, is that the subarachnoid space provides a poor environment for the action of antibody.

There is a considerable body of evidence, however, that acquired immunity is an important factor in man's resistance to meningitis. Specific examples are furnished by *H. influenzae* meningitis which is confined almost exclusively to children between 3 and 18 months of age. Fothergill tested blood from newborns and children of various ages for bactericidal activity against *H. influenzae* and demonstrated that blood from infants under two months and children older than three years killed the organisms regularly, while that obtained from children between these ages was but weakly bactericidal.[68b] Susceptibility of infants to *H. influenzae* meningitis appears to be directly related to the absence of antibody and with the development of active

immunity, presumably on repeated exposure to the organism in the respiratory tract, the host is afforded almost permanent protection from infection with this bacterium. Similarly, the occurrence of meningococcal meningitis is largely determined by the immune state of the host. A case in point is the relatively high incidence of this disease in unseasoned Army recruits. In contrast, clinical cases are extremely rare in sailors at sea where the population turn-over is low.[10a] Acquisition of specific immunity appears to be instrumental in preventing second attacks of meningococcal and hemophilus meningitis which are quite rare. In fact, most recurrent attacks of bacterial meningitis are caused by pneumococcus and, even in this situation, different types are usually incriminated.[81b, 173b]

There is a group of conditions in which man appears peculiarly vulnerable to meningeal infections. Among these are congenital and acquired agammaglobulinemia, in which there appears to be a tendency to develop coccal meningitis,[71a] multiple myeloma, which is frequently complicated by pneumococcal infections including meningitis, and surgical or congenital absence of the spleen, in which pneumococcal meningitis has been a frequent occurrence.[72a, 87a, 158b] In the first two instances, gamma globulin is diminished or functionally defective, and antibody production is markedly impaired. The reason for susceptibility of splenectomized children to infection in general, and meningitis in particular, is not readily apparent. These patients are capable of synthesizing gamma globulin normally, and the concentration of this protein fraction in the serum is normal or elevated. Furthermore, they have a normal response following a provocative dose of diphtheria toxoid.[158b] On the other hand, Rowley demonstrated that splenectomized patients failed to respond with a significant titer following the intravenous administration of sheep erythrocytes, a challenge which was regularly associated with a brisk antibody response in normal experimental subjects.[151a]

It is fair to say, on the basis of these observations, that immune processes are of considerable importance in determining man's susceptibility to meningeal infection. The precise conditions under which these protective mechanisms are operative and the stimuli evoking the appearance of antibody in the nervous system remain subjects for further investigation.

F. *Fever.* Elevation of body temperature does little to disturb the equilibrium between the brain and CSF and the rest of the body unless extreme heights are reached. No change in radioactive phosphorus exchange was found in rabbits with temperatures ranging between 38.2 C. and 41.0 C.[88] Earlier studies indicated that permeability to bismuth and hemolysins to sheep red cells, but not trypan blue, was increased in rabbits with fevers of 42 C.-43 C.[184] In man, elevation of the temperature to 106 F. for

30 minutes on five consecutive days resulted in an increased transfer of bromide from blood to CSF.[130]

Whether the blood brain barrier is permeable to substances which are themselves capable of producing fever has been the subject of considerable study.[141] There is some evidence that endogenous pyrogen, which is presumably the end-product of cellular injury by a variety of pyrogenic stimuli, gains access to the meninges with ease; in contrast, bacterial endotoxins which consist of large lipopolysaccharide molecules gain entry into the CNS only after a considerable delay. King and Wood have interpreted these data to mean that endogenous pyrogen is the substance responsible for activating the thermoregulatory centers,[99] but there are also experiments which suggest that, in the absence of endogenous pyrogen, bacterial endotoxins may affect these centers directly.[141]

COMMENT

It seems clear that a review of this nature will pose more questions than it answers. Without reiterating any specific points, it is still not clear how microorganisms enter the meninges, how infections begin, and what defenses the host mobilizes to combat infection. The unique behavior of various species of bacteria, the mechanisms governing the biochemical alterations in the spinal fluid, and the role of normal constituents of the body fluids in meningeal infection remain unsolved. Entirely shrouded in mystery are the nature of the histochemical and enzymatic defects which must be a part of the profound inflammatory reaction. More pragmatically, the mechanism of death occurring in the face of a bacteriologically sterile spinal fluid remains undefined. If this report has stimulated sufficient interest in these biological phenomena to promote a return of the subject of bacterial meningitis from the historical library to the laboratory, it will have served its purpose.

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EVENTS

YALE MEDICAL SOCIETY

January 19, 1960

THE YOUNG PHYSICIAN AND SOCIALIZED MEDICINE IN BRITAIN. By Harry Eckstein, Center of International Studies, Princeton, New Jersey.

Professor Eckstein began his lecture by noting that from the American view one of the most remarkable points was that the medical profession in Britain actually had taken a leading part in the creation of the British Health Service. Contrary to some prevalent notions, the pressure for its initiation came from two main sources, the medical profession and the Ministry of Health. Differences which arose between these two parties were mainly on technical matters, not the substantive proposals.

Plebiscites taken prior to the establishment of the system showed that the younger men in medicine were overwhelmingly in favor of its adoption. It was suggested that an important reason for their attitude was an economical one, concerned with the financial burden involved in obtaining a practice. The proposed plan included the following general changes.

(1) The traditional method of buying a practice would end.

(2) It would be made more attractive for men to take up practice in regions previously not well supplied with physicians.

(3) Medical incomes would be raised, particularly for the younger men.

(4) Specialist training would be encouraged by adequate payment of physicians still in training.

(5) Better hospitals and facilities would be supplied to encourage both the development and redistribution of specialists.

The achievements of the system have not all been in the direction envisaged. There have been substantial successes. The number of general practitioners and specialists has been increased, and a considerable redistribution has taken place. A most serious failure has been the lack of any large-scale addition to the hospital service, with a consequent shortage of positions for specialist training. This already has produced considerable difficulties for the medical profession and the Government.

A more basic point remains unsolved, namely, the original intent to reduce the economic problems associated with obtaining a new practice. It appears that the established practitioners, exerting pressure through the medium of the British Medical Association, have been able to reduce the amount of financial assistance made available to young men starting their careers.

Professor Eckstein ended his lecture by emphasizing two points:

(1) Socialized medicine does not make a medical career less attractive.

(2) It does seem to lead to a freezing of the intake rate, a circumstance for which responsibility lies mainly with the established physicians.

S. J. F.

ZOOLOGICAL JOURNAL CLUB

February 3, 1960

MECHANISMS OF NORMAL AND ABNORMAL GROWTH IN EXPLANTED CHICK EMBRYOS. By Heinz Herrman, University of Connecticut.

Preliminary investigations suggest that moieties larger than the free amino acids may be protein precursors in the early chick embryo. Initial evidence came from studies of the specific incorporation rate of C^{14}-Glycine into protein of explanted stage 4-7 and 11-13 embryos. Glycine apparently was utilized directly by cells and did not pass into the free amino acid pool, since the free cell glycine maintained a constant specific activity which was lower than that of incorporated protein. Stage 11-13 embryos showed marked increases in DNA and protein-glycine contents when grown on media containing the egg nutrients, especially in cases where large extra-embryonic membrane areas were present. In these explants, however, only a small increase in specific activity of protein-glycine occurred. On the other hand, when grown on synthetic media, explants grew poorly, with no increase in protein-glycine content, although a much greater increase in specific activity occurred. These facts are interpreted to mean (1) that under conditions of poor growth the free amino acid pool is utilized as precursor source for a low level of protein synthesis; and (2) that with good growth the yolk proteins or their breakdown products are being used as precursors, with little labelled material from the free amino acid pool entering into the newly synthesized proteins.

N. K. W.

YALE MEDICAL SOCIETY

February 9, 1960

THE PITUITARY, PINEAL, AND PIGMENTATION. Aaron B. Lerner, Professor of Dermatology, Yale University School of Medicine.

Dr. Lerner and his colleagues have engaged for many years in the study of melanin pigmentation, including the pathways of biosynthesis of the pigment from the amino acid tyrosine and the hormonal mechanisms controlling its production and distribution in the tissues. The latter aspect of this research was presented on February 9th to an appreciative Yale Medical Society audience.

A mechanism other than those involved in *in vivo* pigment synthesis must be invoked to explain the hyperpigmentation in endocrine disorders such as Addison's disease or the hypopigmentation of vitiligo. The movement of pigment granules throughout the cytoplasm of a melanocyte makes the cell appear dark and opaque, whereas clumping of the granules about the nucleus makes the cell appear light. In this manner alpha- and beta-MSH (melanocyte stimulating hormone), two polypeptides isolated from pituitary glands of hogs, have been shown to produce darkening of frog skin by dispersion of pigment granules. These substances also produce darkening of human skin and may do so by a mechanism similar to that which takes

place in frog skin. Amino acid sequences of these compounds have been worked out by Harris and Roos and Harris and Lerner. Porcine alpha-MSH has 13 amino acids. These are identical to the first 13 of the 39 amino acids in porcine ACTH. Porcine beta-MSH, with a somewhat less marked resemblance to corticotropin, has 18 amino acids. Human beta-MSH, currently under study by Dixon and Harris, has been shown to contain 22 amino acids. Potent but not pure preparations of porcine MSH produce darkening of human skin *in vivo*. What remains to be determined is the quantitative darkening action on human skin *in vivo* and *in vitro* using pure porcine alpha- and beta-MSH, ACTH, and the same hormones from human pituitary glands. Hofmann, in the course of his efforts to synthesize ACTH, has accomplished the synthesis of alpha-MSH and several related polypeptides.

The investigation of substances possessing lightening activity has been arduous but rewarding. A readily dialyzable substance, recently isolated from bovine pineal gland, when tested on frog skin, is the most potent lightening agent known. In the investigation of the structure of this hormone Drs. Lerner and Case received invaluable assistance from fellow investigators throughout the country, from the pharmaceutical industry, and from the Department of Pharmacology at Yale Medical School. Designated melatonin, this substance has now been identified as N-acetyl-5-methoxytryptamine. Two important byproducts of this work have been demonstrated: (1) the importance of O-methylation of compounds other than the catechol amines epinephrine and norepinephrine (in this case O-methylation of tryptophan derivatives), and (2) that basic nitrogen (as found in epinephrine, norepinephrine, 5-hydroxytryptamine, and 5-methoxytryptamine, all of which possess lightening activity) is not a requirement for a lightening agent. Acetylation or 5-methoxytryptamine to melatonin, in fact, increases its lightening power many times.

An explanation is thus available for the dark skin of Addison's disease patients: removal of adrenal cortical inhibition of the pituitary allowing increased production of MSH. The role of melatonin in clinical vitiligo is not as clear. Pigmentation remains, however, a field in which biochemistry and clinical manifestations can be actively correlated.

D. M. B.

BOOK REVIEWS

CLINICAL EVALUATION OF NEW DRUGS. By S. O. Waife and A. P. Shapiro, Eds. New York, Hoeber-Harper, 1959. x, 223 pp. $7.50.

The explosive increase in the number of drugs offered for therapy has had many consequences, one of which is to increase the amount of testing that must be done in man. The large number of such studies makes it more urgent that the physician be reminded of his responsibilities in such a program and that he be knowledgeable about the methods for carrying out clinical trials so that the fewest number of patients can yield the greatest amount of valid information with minimum risk, discomfort, and expense. In view of the flood of drugs submitted to clinical trial during the past fifteen years, it is a tribute to the restraint and intelligence of the clinical investigators (with considerable help from man's natural gift for survival) that iatrogenic diseases are not more frequent and that there have not been more unconfirmable therapeutic successes.

Certainly what has been needed is a modern, authoritative, and scholarly account of the clinical evaluation of drugs, a book which suits the need of the investigator and at the same time points out to all physicians the standards by which they must evaluate clinical reports.

Happily, this book has arrived. It has succeeded in presenting in a scholarly way the planning, technique, sources of error, and the variables encountered in evaluating drugs in man. It is emphatically not a "how to" book. Rather, a group of experienced and thoughtful investigators have written chapters on various aspects of drug-testing, presenting broad general principles with pertinent examples to make their points more vivid.

The book is divided into two parts. Part one begins with articles on pharmacological problems in studies on lower animals through the initiation of studies in man. Chapters on experimental design and statistics and placebos follow. Discussions of the ethics of experimentation on human beings, human beings as experimental subjects, the investigator himself, and the training of an investigator complete the first part of the book. The second section comprises six chapters on a selected group of disorders: infectious diseases; cardiovascular disorders; gastrointestinal diseases; nutrition and metabolism; endocrinologic problems; and neuropsychiatric disorders. The volume ends with a short article on the problems of publication. All the chapters in the second section are brief but they serve to demonstrate the common experiences and the wide areas of agreement of the authors.

Contributors are Karl H. Beyer, Elmer L. Severinghaus, Louis Lasagna, Paul Meier, Henry K. Beecher, William B. Bean, Stewart Wolf, Dwight J. Ingle, Harry F. Dowling, Thomas E. Machella, J. Lester Gabrilove, Nathan S. Kline, and both editors. The book is exceedingly well written.

All physicians and medical students should read this, not once but several times. It will never fail to sharpen the reader's critical sense. It not only shows the complexities inherent in evaluating therapy but is itself sobering prophylaxis for the present therapeutic binge.

JACK PETER GREEN

PERSPECTIVES IN VIROLOGY. Morris Pollard, Ed. New York, John Wiley & Sons, 1959. xix, 312 pp. $7.00.

As the name implies, this book attempts to give a broad picture of new developments in the field of virology, and in this it succeeds admirably. The volume represents the proceedings of a symposium, dedicated to the late Dr. F. R. Beaudette, an outstanding virologist in the field of animal virus diseases. A representative sample of new discoveries and new problems in virology—in the basic science area, epidemiology, and immunology—is presented. The participants in the symposium are well-recognized scientists from Europe and the USA who present their material in a straightforward and readable manner.

Several papers are devoted to the biochemical aspects of virus cell relationships and the mechanisms of viral reproduction. Fraenkel-Conrat describes experiments with tobacco mosaic virus, which is known to consist of an inner cylinder of ribonucleic acid, and an outer cylinder of protein. The nucleic acid alone was found to be able to perform all viral functions in the absence of the protein, to infect the host cell and transmit all the genetic information required for the production of both progeny virus nucleic acid and protein. Schäfer reports on work with fowl plague, using it as a model for studying the composition and mode of multiplication of myxoviruses. Using the fluorescent antibody technique of Coons and Kaplan, the steps in the formation of new virus in infected cells was followed.

A number of papers are concerned with the relation of viruses and cancer. Kilham reports a series of experiments on transformation of the benign Shope fibroma virus into highly fatal myxoma virus in tissue culture, using heat killed preparations of myxoma virus as a transforming agent. The extraordinary range of viral tumors, from avian leukosis to the various mammalian tumors and the recently discovered mouse leukemia viruses, are reviewed by Beard, who concludes that "it is impossible to conceive that the phenomena of viral neoplasia are unique to animals other than man," and notes that a concerted effort to find analogous relations between viruses and rumors in man is indicated. The demonstration of antibody formation and classical immunological reactions associated with the mouse leukemia virus reported by Friend, adds another important chapter to the subject of viruses and malignancy. Stewart and Eddy summarize their work with the remarkable SE polyoma virus, which also induces antibody formation, and therefore behaves like an "ordinary" virus, in spite of its somewhat spectacular characteristics.

The common viruses, influenza, measles, and the host of new entero, adeno, and myxoviruses, are also discussed in a series of excellent papers. In "Asian Influenza: A Challenge to Epidemiology," Andrewes reviews the possible origin of the recent epidemic from an animal reservoir in China. Enders *et al.* brings the measles story up to date with work on the attenuation of this agent by repeated passage in tissue culture and the possible use of the attenuated virus as an active immunizing agent.

The discussions by participants in the symposium are often as long as the papers and contain many provocative points. It is impossible for one small book to cover all of current virology, but this book accomplishes its

purpose well in that it brings together much new information and lively discussion on a variety of aspects of the subject and gives the reader a true working perspective on this rapidly expanding field.

DOROTHY M. HORSTMANN

THE SCIENCE OF BIOLOGY. By Paul B. Weisz. New York, McGraw-Hill Book Co., 1959. 796 pp. $7.95.

Instructors and students alike will find Weisz's *The Science of Biology* artfully presented and intellectually stimulating. The format, common offender in many biology texts, is here logical and original in many respects. Following an orientation for the student on the facets and ethics of science, the author proceeds with the beginnings of chemistry and the origin of cells, cells to multicellularity, organisms and their association with one another, and finally, their habitats. He then returns the reader for a closer view of protoplasm, its chemistry, function, and organization in the plant and animal body. Beyond this, the attributes of living systems—metabolism, steady-state, reproduction and adaptation—are discussed and compared in the plant and animal. The mechanics of adaptation, including the genetic phenomenon of mutation, conclude quite naturally with evolution and descriptions of phyla. The reader is left with a sense of having viewed the entire "struggle" to modify form within the confines of existence.

Many excellent and well-selected photographs are included; those from electron microscopy are especially appropriate at this time. Concise and aesthetically pleasing diagrams are amply distributed throughout. The finesse with which the author has used these diagrams to summarize data or to contain a theme is highly commendable. Although they may appear to be simple—this is a misconception. To properly understand them, one must first read the text. This is a valuable asset in the training of students who might otherwise prefer to learn solely by "visual aid."

The sections on energetics (metabolism and energy transfer cycles) are thoughtfully and thoroughly treated and are essentially up to date. While they are not always easy to follow, they do include, nevertheless, very pertinent information. It is welcome to find them in this kind of text.

The reviewer was deeply impressed by the grace and clarity with which the author integrated an assortment of facts in presenting his central theme or principle. In this, his purpose, he has been most successful. The chemical, physical, anatomical, and functional aspects of a problem are often combined without burying the prospective in an excess of explanatory information. This means, *a priori,* that the student may have to look elsewhere for information regarding a point he does not understand. Some may regard this a weakness. However, the express purpose of the book has depended upon the presentation of significant facts, integrated for the support of biological principles. To make such principles understandable, or indeed even presentable, within the space of a text, some supportive information must be sought in other areas.

Since the author has the ability not only to integrate information but to stimulate thought, it was disturbing to find that he did not introduce con-

troversy where it properly belonged. In his uni-directional approach to the origin of cells, and in other areas as well, it was obvious that the data included, and the interpretation, depended on "hindsight." He may also be criticised for overlooking his principles of the scientific method (admirably presented in an earlier section) by appending finality to a body of selected data. In other instances where information is either lacking or opinions are severely divided the "conspicuous-by-absence" fault must apply. In discussions of high-energy phosphate sources in the citric acid cycle or mechanisms of protein synthesis, for example, a few qualifications to the student would seem advisable, especially since such problems are fundamental to life and considerable space had been devoted to them. Hence, the reviewer would have proferred to see some of the unknown elements of science presented in proper perspective, which the talents of the author would permit him to do. Were it not for the question marks between the periods there would be no scientists, and even for thoughtful students, finality in the method of presentation dispels intrigue.

PATRICIA F. KNIGHT

A TEXTBOOK OF GENERAL PHYSIOLOGY. 2d ed. By Hugh Davson. Boston, Little, Brown and Company, 1959. xvi, 846 pp., ill. $14.50.

In the second edition of this remarkable and highly successful textbook of general physiology, Professor Davson performs a commendable task in presenting a most lucid account of the principles of physiology. The success of the present work is primarily due to his ability to be both clear and concise. He defines general physiology as the study of those features that appear to be common to all forms.

Davson's new work is divided into six sections: (I) The Structural Basis of Living Matter, (II) Transformation of Energy in Living Systems, (III) The Transport of Water and Solutes, (IV) Characteristics of Excitable Tissue, (V) The Mechanism of Contraction of Muscle, and (VI) Light—Its Effect On, and its Emission by, the Organism. The section on neurophysiology is very interestingly developed and is certainly a step forward in the beautiful integration of neuroanatomy (including cytology), neurophysiology, and neuropharmacology.

This new and revised textbook, which is considerably larger than its predecessor, will appeal to a wide and divergent community of scholars. First and foremost, it is oriented for the advanced student of physiology, both general and comparative. Secondly, it presents an enormous amount of highly specific and accurately detailed data for students of medical physiology. Finally, the research worker in physiology will realize much profit by arming himself with an authoritative analysis of the latest concepts of physiology described.

The author presents an up-to-date account of the principles of general physiology and in doing so makes liberal use of current references. Of singular significance is his evaluation of the current references cited in his new edition. Giving added strength to the second edition are his new chapters on the sensory response, excitability of cardiac muscle, and electrical activity of smooth muscle. His chapters on cerebrospinal fluid, utilization

of chemical energy, and functional changes in the cell and their mechanisms have been extensively expanded and brought up to date.

This is truly a remarkable book—one to be recommended without any reservation. It is hoped, however, that in future revisions the author will give us the extended benefits of his scholarly concepts by including a chapter on reproductive mechanisms.

JOSEPH THOMAS VELARDO

THE PHYSIOLOGY AND TREATMENT OF PEPTIC ULCER. By J. Garrott Allen and others. Chicago, University of Chicago Press, 1959. 236 pp. $7.50.

This book contains a report of work done at the University of Chicago and is dedicated to Dr. Lester R. Dragstedt. Inasmuch as the book describes "experiments selected from a large number designed and conducted by Dr. Lester R. Dragstedt," it does not attempt to cover the gamut of physiological considerations. As might be expected, the role of the vagus and of the antrum in controlling gastric secretion is emphasized, while the possibility that there is an adrenal phase to gastric secretion is given little attention. The title of the monograph, *The Physiology and Treatment of Peptic Ulcer,* asks for a more universal approach.

Each chapter of the book written by such authorities as Henry N. Harkins, Paul C. Hodges, Joseph B. Kirsner, and Walter L. Palmer, taken by itself, is informative. Unfortunately, "no attempts have been made to reconcile different points of view." This may leave the uninitiated reader uncertain; a more unified approach would have been desirable.

As an expression of loyalty to Dr. Dragstedt the book is appropriate, but one awaits with greater interest the anticipated publication of Dr. Dragstedt's own monograph on peptic ulcer which by its nature presumably will express a strong unitarian view.

H. M. SHAPIRO

THE CIBA COLLECTION OF MEDICAL ILLUSTRATIONS, Volume 3, DIGESTIVE SYSTEM, Part I, UPPER DIGESTIVE TRACT. By Frank H. Netter, New Jersey, Ciba Pharmaceutical Products, Inc., 1959. ix, 206 pp. $12.50.

The book contains 172 full-color plates illustrating the anatomy, the diseases, and some functional and diagnostic aspects of the upper intestinal tract. It is fortunate that the book is sold at cost because the color reproductions are of the highest quality on expensive gloss paper. Nine well-known authorities acted as consultants to Dr. Netter. The teamwork between the consultant and artist has resulted in unusually clear, accurate illustrations and descriptive text. Most of the illustrations depicting the physiological and diagnostic aspects are unique in their presentation, and often one plate puts across points to the reader which would take many written pages to accomplish.

The consultants, artist, and editor have tried to supplement graphically the standard reference works rather than to replace them. They have done this quite well. As a teacher of anatomy I have found that the medical

students derive much benefit from a book of this type. As a supplement to their standard texts it not only aids them in understanding structure but stimulates their studying of anatomy by integrating physiology and emphasizing the practical application of such knowledge. A book of this type allows a busy physician to review in a short time the essentials of normal anatomy of various structures of the body and at the same time keep abreast of some of the latest methods of diagnosis and treatment when the structures are diseased.

E. S. CRELIN

PRINCIPLES OF RADIATION DOSIMETRY. By G. N. Whyte. New York, John Wiley and Sons, Inc., 1959. v, 124 pp. $7.00.

The purpose of this book is to describe the fundamental physical principles involved in the dosimetry of charged particles, x-rays, and neutrons. The book starts by discussing the properties of radiation, the intensity of radiation, and the amount of energy absorbed per unit mass in the irradiated material. The author continues with consideration of the mechanisms whereby radiation fields interact with matter, removing energy from the beam of radiation and the absorbtion of energy by various materials. These fundamentals are then applied to the problem of measurements on radiation fields, and their effects on the measurements of radiation intensity, exposure dose, and absorbed dose are considered. The author discusses the differences between exposure dose and absorbed dose. The book closes with a discussion of the techniques for primary standards of radiation dosimetry, secondary standards, and the techniques used for routine dosimetry.

While the examples given in the book are chiefly concerned with biological and medical applications, the principles are discussed quite generally and apply to all problems in the dosimetry of charged particles, x-rays, or neutrons. Only a small amount of experimental data is included so the reader must refer to the bibliography for the information necessary in the application of the subject. It is the absence of such data, however, which makes the book valuable. The reader may move quite rapidly through the various subjects and obtain a clear picture of the field of radiation dosimetry and its relationship to the fundamentals of physics. To do this, the language of mathematics and especially calculus must be used at various points throughout the book. The reader unfamiliar with calculus can still obtain an understanding of the physical aspects of radiation dosimetry, although some of the completeness of the picture may be lost.

Each chapter contains a bibliography of the more important references and should be useful in leading to the more specific details and data needed in problems of dosimetry. The book forms an ideal starting point for the individual embarking on problems involving radiation dosimetry as well as those who have been familiar with the subject in the past and need to refresh their viewpoints in terms of the changes which have taken place in the field in recent years.

J. EDWARD DONNELLAN, JR.

BLOOD GROUPS. K. L. G. Goldsmith, Scientific Editor. British Medical Bulletin, vol. 15, no. 2. London, Medical Department, The British Council, 1959. 174 pp. $3.25.

A glance at the table of contents of the British Medical Bulletin on Blood Groups reveals the complexity of the subject. It is a credit to the editor that he assembled a group of authors who not only know what to say but how to say it.

This volume, short and to the point, provides a review of the newer developments in immunohematology which is suitable for students and practicing physicians alike. It consists of 15 chapters, each written by a research worker in the field. The topics covered range from The Biochemistry of Human Blood Group Substances to Management of Hemolytic Disease of the Newborn. Each chapter is followed by a well chosen list of pertinent references.

The chapter on Relative Importance of Blood Group Antibodies, in particular, provides a concise evaluation of those antibodies which are important and the reasons for their importance. It should be required reading for all people who use blood transfusion in practice. It is also highly recommended for those who desire an accurate and readable review of modern blood grouping.

JOSEPH R. BOVE

THE NEW PSYCHIATRY. By Nathan Masor. New York, Philosophical Library, 1959. 155 pp. $3.75.

In this slim volume, Dr. Masor presents a virulent attack on psychoanalytic schools of thought and presents his own ideas on the biochemical etiology of mental disease, unsupported, however, by any objective experimental work on his part. The tone of the book is one of sarcasm.

The author, using subjective opinions only, espouses the idea that all psychoanalytic thought, especially that of Freud, is completely erroneous, and that all mental illness can be explained on the basis of altered biochemistry. However, he does not specify in which ways current research supports his ideas, i.e., he states that it has been found that neuraminic acid levels are lower in the spinal fluid of children and schizophrenics than in that of normal adults and then closes his discussion of the topic with the statement that chemical immaturity may correlate with psychological immaturity. Equally unfounded is his critique of accepted psychoanalytic theory; he denies the role of the unconscious in mental disease, substituting what he terms a more "rational" approach, unfortunately unsupported by documentary evidence. This consists of the idea that emotions per se are important in the functioning of the unconscious, whereas actual content and its relation to the ego (the existence of which he denies) is unimportant.

The book contains much information and is full of statements such as: "Psychoanalysis, in its theoretical consideration, is opposed to good common sense," and "It is my belief that dreams have no meaning precisely for the enumerated reasons that Freud offered why they do have meaning."

The author misstates and oversimplifies much of Freudian theories, and in stating his own ideas does so with assurance but no documentary evidence. There are a number of factual errors in the book, such as the notion that folic acid cures pernicious anemia. The grammar and style are awkward in many places, and it is written in a nonmedical style.

To summarize, this short work represents the attempt of a general practitioner to substitute his own ideas concerning mental illness for those of established psychoanalytic and psychiatric thought. It is not very successful.

D. W. B.

A History of Biology to About the Year 1900. 3d and rev. ed. By Charles Singer, London and New York, Abelard-Schuman, [1959]. xxxvi, 580 pp. $6.00.

The title of this standard work precisely describes its contents. The book is an internal history of biology from the time of Hippocrates through the 19th century; Dr. Singer does not describe the relationships between biological discovery and social milieu in which the discoverers worked. For instance, the reader will not find here a discussion of the influence of 19th century liberalism on Darwin's ideas.

Although this printing is described as the "Third and Revised Edition," an owner of the revised edition of 1950 will not have to purchase this volume. The new edition has an additional numbered page of introductory matter because of the "Preface to the Third Edition," but it has the same number of text pages according to the Table of Contents which, however, gives the wrong page for the Index—there are two more text pages including a new Epilogue. Many pages at the beginning of chapters are exactly like those of the 1950 imprint.

Some idea of the extent of revision can be gained from the first change. In 1950 the last sentence of the first paragraph of chapter one read, "They [the Greeks] came from the north and settled in their new home toward the end of the second millennium before Christ." In 1959 this sentence is, "They had settled there about the beginning of the second millennium before Christ." The revision is more accurate; they came from the East.

Here and there a chapter gains a page of text so that the last now begins on page 548 while it started on 540 in 1950. But to limit the page increase to two and to make room for a four-page Epilogue the last two chapter sections on the gene have been eliminated thereby strengthening the dike at 1900 if doing nothing else.

In other words, this edition is no real revision, but it is the best history of biology available.

FREDERICK G. KILGOUR

The Cytopathology of Virus Infection. By Robert Love and others *Annals of the New York Academy of Sciences,* vol. 81, art. 1, pp. 1-24. New York, 1959. $4.50.

In old-fashioned virology, interest often centered on various types of inclusion bodies and great diagnostic significance was attached to them.

More recently, in the hurly-burly of expansion that has followed the development of new virus culture methods, pathological changes in virus infected cells have frequently been neglected. Even the electron microscope was used at first almost exclusively in the study of extracellular virus. Now, in this symposium, sponsored by the New York Academy of Sciences, Dr. Robert Love has brought together a group of scientists who have continued work in this painstaking field. Older techniques of histology and cytochemistry have been applied to various new systems, and several new techniques are reported which hold great promise for the elucidation of virus growth mechanisms and the patterns of cell response.

Electron microscopy has been extensively used in the study of sectioned infected cells and has yielded considerable information in those systems, such as pox and salivary gland virus infection, where distinctive particles occur. Studies by Dr. Lepine and his group using cinematography, and other studies using phase microscopy help to tie observations on fixed preparations with the transformations that occur in living cells. However, the newest and most promising reports are those that describe the chemical identification of components in infected cells by means of fluoroscein labelled antibody, micro-spectrography and the fluorescent stain, acridine orange. It is only a pity that the striking colored pictures of acridine orange stained cells that were shown by Dr. Niven could not be reproduced in this volume.

Although this is a technical publication on a specialized subject, its descriptive nature and many illustrations, together with the fundamental import of the results make this a volume of more than usual interest to the general scientific reader. The descriptions of new techniques and the new information on mechanisms of virus growth make it a useful reference for virologists. The sometimes neglected approaches to virology that are represented in this volume will doubtless yield important information in our search for the basic processes of biological replication.

FRANCIS L. BLACK

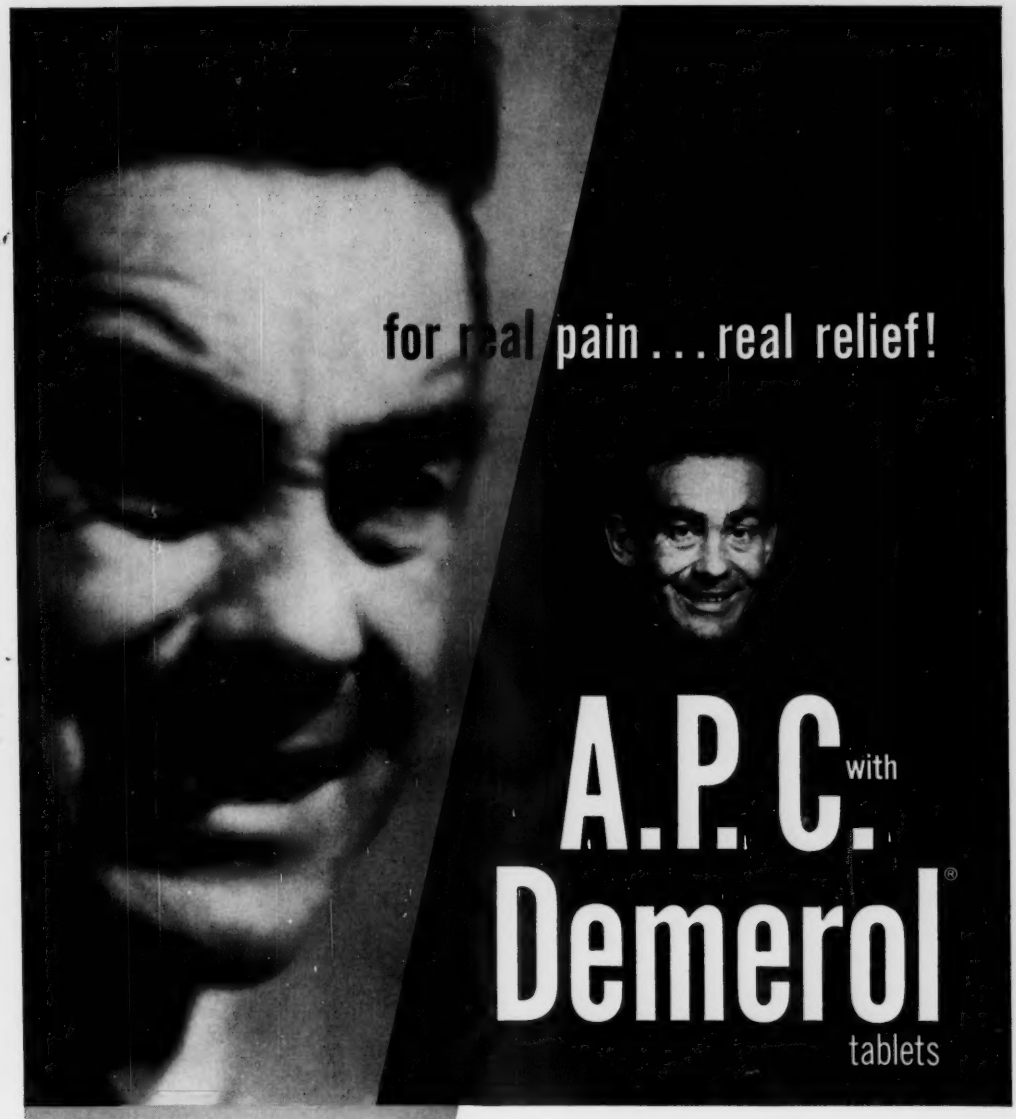
COMPARATIVE ENDOCRINOLOGY. Proceedings of the Columbia University Symposium on Comparative Endocrinology held at Cold Spring Harbor, New York, May 25 to 29, 1958. Aubrey Gorbman, Ed. New York, John Wiley & Sons; London, Chapman & Hall, 1959. xx, 746 pp.; illus. $15.00.

Comparative endocrinology is a relatively neglected but rich and exciting field. Something is known of the basic endocrine functions and relationships of fowl and of certain amphibians, but very little is understood regarding reptiles, fishes, and the invertebrates. The varieties of reproductive behavior manifested by the fishes alone, behavior believed to be primarily under humoral control, constitute only one of the areas within the broad dimensions of comparative endocrinology.

The organization by Dr. Gorbman and his committee of an international symposium in this field was therefore of importance. The contributions and discussions heard at the symposium are now available in a well-edited volume. Forty-three reports by specialists, the appended bibliog-

raphies, and the discussion make available a whole series of useful summaries, much to be recommended to anyone interested in the comparative aspects of endocrinology and neuroendocrinology. Subjects range from heterotopic thyroid tissue in fish to viviparity in frogs to the comparative physiology of the adrenal cortex and endocrine pancreas. Unfortunately, the subject index is only six pages long, a difficulty partly alleviated by a separate "Index of Animal Names." A pleasant innovation is the inclusion of a series of snapshots of numerous scientists present at the symposium.

THOMAS R. FORBES



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